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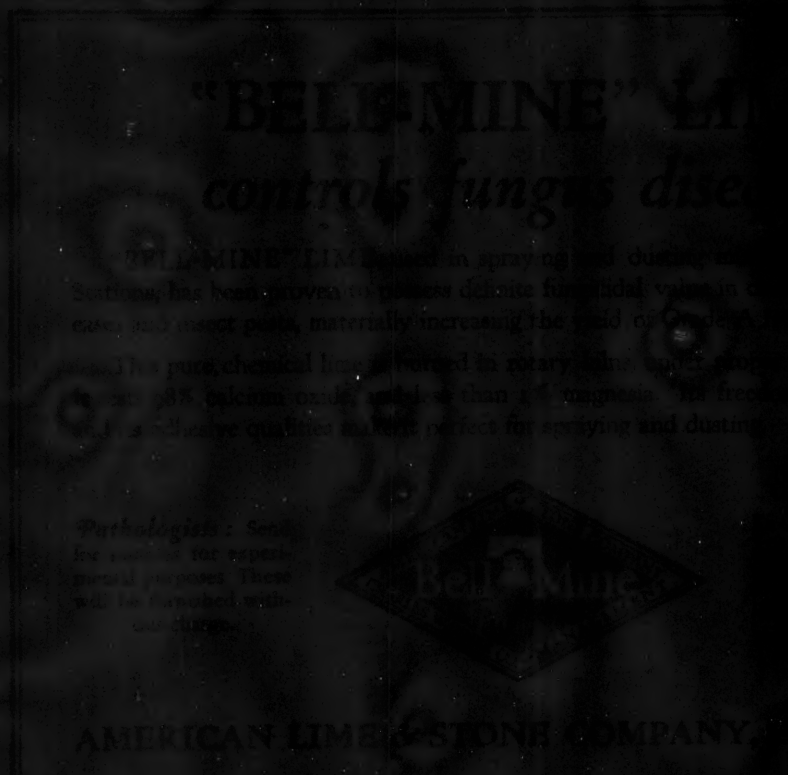

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PHYSIOLOGY AND PARASITISM OF SCLEROTIUM ROLFSII SACC.[1]

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INTRODUCTION

Throughout the warmer sections of the United States, *Sclerotium rolfsii* is one of the most destructive of soil-inhabiting fungi. It attacks a great variety of cultivated and wild plants in the field, killing the plants outright. It is also a common cause of decay in stored roots and vegetables and of cucurbit and other fruits that rest upon the soil. Very often it destroys planted seeds and seedlings and has several times been reported as a serious pest in nurseries.

Plants once attacked by the fungus are nearly always killed; but, unlike the wilt-producing species of *Fusarium*, which usually attack every plant within an infested area, the attack of *S. rolfsii* may be very irregular. In pepper fields the loss may vary from a few scattered plants to as many as 75 per cent. In soil containing large quantities of undecayed organic matter, every plant may be killed; yet susceptible plants have been grown free from attack over long periods in soil containing numerous sclerotia of the fungus. This latter statement may be illustrated by a brief report of observations on a series of 24 pot cultures of pepper plants grown in a compost soil in the greenhouse for one year. Each pot was inoculated with a culture of the fungus on sweet potato plug as soon as the plants were well started. The fungus spread and killed several plants in each pot during the following two weeks. It then formed numerous sclerotia and ceased growth, remaining dormant over a period of six months. Although the sclerotia were viable, they did not germinate. Finally the plants became infested with aphids and, with the accumulation of detritus from the aphids, the fungus entered another period of growth and killed most of the remaining plants. This was followed by a dormant period of three months, and, finally, a third period of growth.

No practical method of control has yet been developed. Crop rotation is of some value in reducing losses; but, on account of the very wide range of

[1] Paper number 23, Journal Series, Georgia Agricultural Experiment Station.

host species, it is difficult to plan a rotation to rid the soil of this fungus. However, the unusual geographical distribution of the fungus, together with some peculiar aspects of its behavior, has suggested that a thorough knowledge of its physiology might lead to an understanding of its wide host range, its limited geographical range, and possibly to a solution of the problem of its control.

The most obvious factors which might affect its geographical range are temperature and soil reaction. The first investigation, therefore, was directed principally toward (*1*) determination of temperature relations of the fungus, (*2*) relation of reaction of substratum to growth, (*3*) reaction changes in the substratum during growth, (*4*) relation of various organic nutrients to growth, (*5*) metabolic products of the fungus, (*6*) relation of the metabolic products to parasitism, and (*7*) relation of fungous hyphae to the host tissues.

MEDIA

As *S. rolfsii* is a soil-inhabiting fungus, often appearing on plants in soil containing comparatively little organic matter, it seemed probable that it might require considerable quantities of inorganic nutrients for its best growth. However, preliminary trials on Czapek's solution did not result in very satisfactory growth. Following this, a series of cultures was grown on six synthetic media, variations of Czapek's solution and Richard's solution E., as given by Zeller, Schmitz, and Duggar (24). For comparison with these synthetic media, the following were used: bean decoction, decoction from pepper pods, beef-extract-peptone broth, Dunham's solution, 1 per cent peptone solution, and 2 per cent egg albumen solution.

On none of the synthetic media tried did the fungus appear to grow normally. The growth was slow, and few or no sclerotia were formed. When either peptone or egg albumen was added to replace sugar, the growth was much better and sclerotia formed more abundantly, but only slightly better than on the same concentrations of peptone or egg albumen in distilled water. The addition of sodium chloride to the peptone solution seemed to have little effect. The fungus grew rapidly and luxuriantly, and formed abundant sclerotia on the vegetable decoctions and the standard beef-extract-peptone broth. The proportion between mycelium and sclerotia and the general appearance of the growth was quite similar to that seen on various fruits and vegetables in the field. The growth on broth was considerably more abundant than that on a 1 per cent peptone solution. This was doubtless due to the mineral elements in beef extract.

Since some organic nitrogen seemed necessary for normal growth of the fungus, the standard beef-extract-peptone broth[2] was adopted as the basal

[2] Beef-extract 3 grams, peptone 5 grams, distilled water 1 liter.

medium for the study of hydrogen-ion relations, and beef-extract-peptone agar[3] for the study of temperature relations.

The beef extract, peptone, and sugars used were those especially prepared for bacteriological work by the Digestive Ferments Company. The other chemicals were of the highest commercial purity. Pyrex glassware was used for all liquid cultures. It was cleaned with alkali and acid, thoroughly washed and rinsed twice with distilled water at the beginning of each experiment. The distilled water was steam distilled in a Stokes Automatic Apparatus, lined throughout with block tin.

All hydrogen-ion determinations were made colorimetrically, using standard indicator solutions and a comparator of the Army Medical School model.

TEMPERATURE RELATIONS

For the study of the temperature relations of the fungus, the rate of growth of mycelium on standard beef-extract agar plates was adopted as the criterion. The results thus obtained were checked also against the length of time required for germination of sclerotia.

Standard petri dishes with bottoms as nearly flat as possible were selected and sterilized. Ten cc. of sterile agar was then poured into each plate, which, after hardening, was inoculated with a single sclerotium, placed near the center of the plate. These were held for 48 hours at approximately 25° C. The limits of the colony in each plate were then marked with ink on the bottom of the plate. The plates were distributed to incubation chambers and incubated for 48 hours at the various temperatures. At the end of 48 hours the plates were removed and the average increase in diameter of each colony measured.

Five strains of *Sclerotium rolfsii* (pepper, Irish potato, peach, Cedrus, and snapdragon strains) were used. Two plates of each strain were placed at each of the temperatures. The results for each of the temperatures are

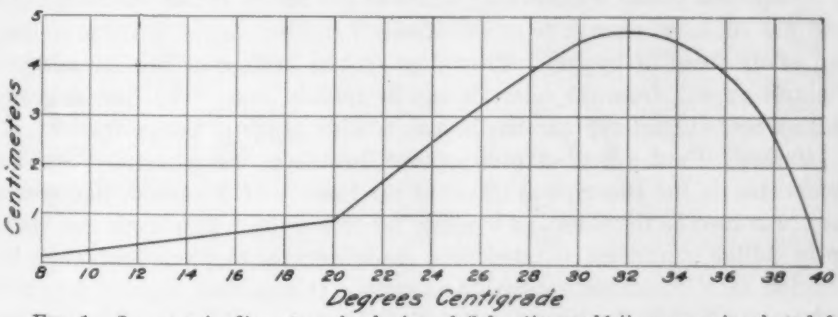

FIG. 1. Increase in diameter of colonies of *Sclerotium rolfsii* on agar incubated for 48 hours at temperatures indicated.

[3] Beef-extract 3 grams, peptone 5 grams, agar 15 grams, and distilled water 1 liter.

therefore based on the average measurements of ten colonies. Several lots were incubated at temperatures near the optimum and maximum, and the lower temperatures were checked once. The temperatures used were −10, −2, 3 to 5, 8, 10, 15, 20, 25, 27, 30, 33, 35, 37, 39, 40, 41 and 42° C. The results are shown graphically in figure 1.

For 10 days the fungus did not grow at all at temperatures below freezing or at 3 to 5° C. At 8° C. growth was very slow, only an average increase of 1.46 mm. in diameter in 48 hours; and the average rate over the 10-day period was approximately the same. Fresh sclerotia had not germinated in this chamber at the end of ten days. At 10° C. the sclerotia had germinated at the end of 7 days, and at 15° C. in 4 days. The fungus grew well at all temperatures between 20° and 37° C. Some increase in diameter of the colonies was obtained up to 40° C., but growth soon ceased, and no sclerotia germinated at temperatures above 37° C. The mycelium was not killed by 48 hours exposure to 42° C. When the plates were removed to a lower temperature, vigorous growth soon started again. The minimum, optimum, and maximum temperatures are 8°, 30° to 35°, and 40° C., respectively, with 37° C. the apparent maximum for normal growth of the fungus.

The vegetative mycelium was killed in all cases by exposure to −2° C. for 24 hours, but the mature, ungerminated sclerotia were not killed. Similar results were obtained at the end of 48 hours exposure at −2° C., and at the end of 24 and 48 hours exposures at −10° C. The plates were removed from the freezing chamber and the agar allowed to thaw at room temperature (about 10° C.), then placed in an incubation chamber at 25° C. and left for 48 hours. The mycelium therefore cannot survive freezing, but the ungerminated sclerotia are much more resistant. The effect on sclerotia of alternate freezing and thawing was not investigated.

RELATION OF H-ION CONCENTRATION TO GROWTH

Sclerotium rolfsii is admirably suited to a study of H-ion relations. The sclerotia are large enough to admit of easy handling, fairly uniform in size, and easily freed of hyphae. They float on the surface of liquids, and the slightest growth from the sclerotia can be readily seen. The fungus grows well on beef-extract-peptone media over a wide range of concentrations.

Methods Used.—Beef-extract-peptone broth, as recommended by the Committee on the Descriptive Chart of the Society of American Bacteriologists, was used as the standard medium for this study. The broth was made up in 3-liter quantities, titrated, and made neutral to phenolphthalein by addition of N/1 sodium hydroxide solution. It was then divided into 250 cc. lots, and N/1 HCl solution or N/1 NaOH solution added to make each lot acid or alkaline according to Fuller's scale. In this way solutions were made up −5, −4, −3, −2, −1, neutral, +1, +2, +3, +4, +5, +10, +15, +20, +25,

+30, +35, +40, +45, +50, +60, +65, +70, +75 and +80. Fifty cc. of solution were placed in each of five 100 cc. Erlenmeyer flasks and sterilized. One flask of each set of five was inoculated with a single sclerotium of a strain of *Sclerotium rolfsii* isolated from pepper stems, one with a strain from Irish potato tuber, one with a strain from peach seedlings, and one with a strain from seedlings of *Cedrus deodara*. The fifth was held as a blank for determination of H-ion concentration at the end of the incubation period. All were incubated six weeks at a temperature of 25° C.

The four strains of the fungus were selected from a large number of stock cultures because of the differences noted in the general characters, especially in the size and color of sclerotia. For the inoculations, mature sclerotia were selected from cornmeal agar cultures of uniform age and were handled with a platinum loop to avoid injury. No attempt was made to determine the amount of growth produced in this series because the mycelium extended up the sides of the flasks so that all growth could not be removed. The results are shown in table 1.

TABLE 1.—*The effect of six weeks growth of four strains of Sclerotium rolfsii on the H-ion concentration of broth adjusted to different initial H-ion concentrations*

Initial acidity, Fuller's scale	H-ion concentration after six weeks					Average pH of cultures
	Check. Not inoculated	Culture from pepper	Culture from potato	Culture from peach	Culture from *Cedrus*	
Neutral	8.30	No growth	No growth	No growth	4.10	4.100
+ 1	8.10	4.10	4.0+	No growth	Contaminated	4.050
+ 2	7.90	4.10	4.0+	4.10	4.20	4.100
+ 3	7.50	4.10	4.0+	4.20	4.20	4.150
+ 4	7.20	4.10	4.0+	4.00	4.10	4.150
+ 5	6.80	4.00	3.80	4.10	4.00	3.975
+10	5.00	4.00	3.80	4.10	4.00	3.975
+15	4.10	4.00	3.80	4.10	4.10	4.000
+20	3.60	4.20	4.20	4.40	4.00	4.200
+25	3.20	4.10	4.00	4.10	4.00	4.050
+30	2.80	4.00	3.90	4.10	4.00	4.000
+35	2.20	4.20	3.80	3.90	4.20	4.025
+40	1.90	4.00	3.20	3.50	3.70	3.600
+45	1.70	4.40	3.40	3.80	4.20	3.950
+50	1.60	4.20	3.20	3.20	3.70	3.575
+55	1.5+	3.20	2.80	2.80	3.20	3.000
+60	1.50	3.00	2.00	2.10	2.60	2.240
+65	1.5−	1.70	1.60	1.50	1.80	1.650
+70	1.45	1.50	1.45	1.50	1.50	1.490
+75	1.4−	1.45	1.40	1.45	1.50	1.450
+80	1.35	No change				No growth in any strain

There was a slight but constant difference in the relation of some of the strains to H-ion concentration. The Cedrus strain grew in broth neutral to phenolphthalein (pH 8.3); whereas none of the other strains grew beyond pH 8.1. The peach form did not grow beyond +2, pH 7.9. On the acid side, all grew in +75 (pH 1.4−) but not in +80 (pH 1.35). Growth appeared earliest in +10 and +15 (pH 5.0 to 4.1), but the total amounts of mycelium present at the end of six weeks were approximately equal over the range from neutral to about +50 (pH 1.6). In the more acid lots, +55 to +75, growth was slow in starting; the total amounts of mycelium were small; and usually few or no sclerotia were produced, the last character again varying with the strain of the fungus.

On beef-extract-peptone agar the range was somewhat wider. Growth occurred at −2 and +85 Fuller's. The exact H-ion concentrations were not determined.

In broth + 1 per cent NaCl the range was somewhat narrowed. The Cedrus strain grew from +3 (pH 7.5) to +70 (pH 1.4), the pepper strain from +4 (pH 7.2) to +75 (pH 1.4−), the potato strain from +4 to +70, and the peach strain in +4 to +60 (pH 1.5). The explanation of this narrowed range doubtless lies in the stronger buffer action of the NaCl broth, which would of course make the medium less readily adjustable to an H-ion concentration favorable for growth of the fungus.

Addition of saccharose to the beef-extract-broth affected quite materially the tolerance of the fungus for both hydrogen- and hydroxyl-ions. The broth was made, neutralized and the normal solution of NaOH or HCl added. It was then flasked, 25 cc. per flask, and sterilized. A 7 per cent solution of saccharose in distilled water was sterilized separately and 10 cc. added to each flask of sterile broth, making 35 cc. of slightly diluted broth containing 2 per cent saccharose.

Growth was obtained in all solutions over the range from −5 (pH 8.8) to +60 (pH 1.4+). The range of tolerance was extended on the acid side and shortened on the alkaline side. Growth started first in solutions of pH 1.6 to 1.5, but subsequent growth was most rapid and greater at lower initial concentrations.

GROWTH OF FUNGUS AND CHANGE IN REACTION PRODUCED ON VARIOUS MEDIA

By reference to table 1, the constancy of the hydrogen-ion concentration may be noted at once. In all broth cultures where good growth of the fungus was obtained, regardless of the initial reaction, the final concentration was near pH 4.0. In the broth with the more acid initial reaction the H-ion concentration was reduced in proportion to the growth of the fungus; in those with an initial reaction between pH 4.0 and the lowest concentra-

tion at which growth was obtained, the H-ion concentration was increased.

Evidently both acid and alkaline substances were among the metabolic products of the fungus on broth; but, in saccharose broth, acids were evi-

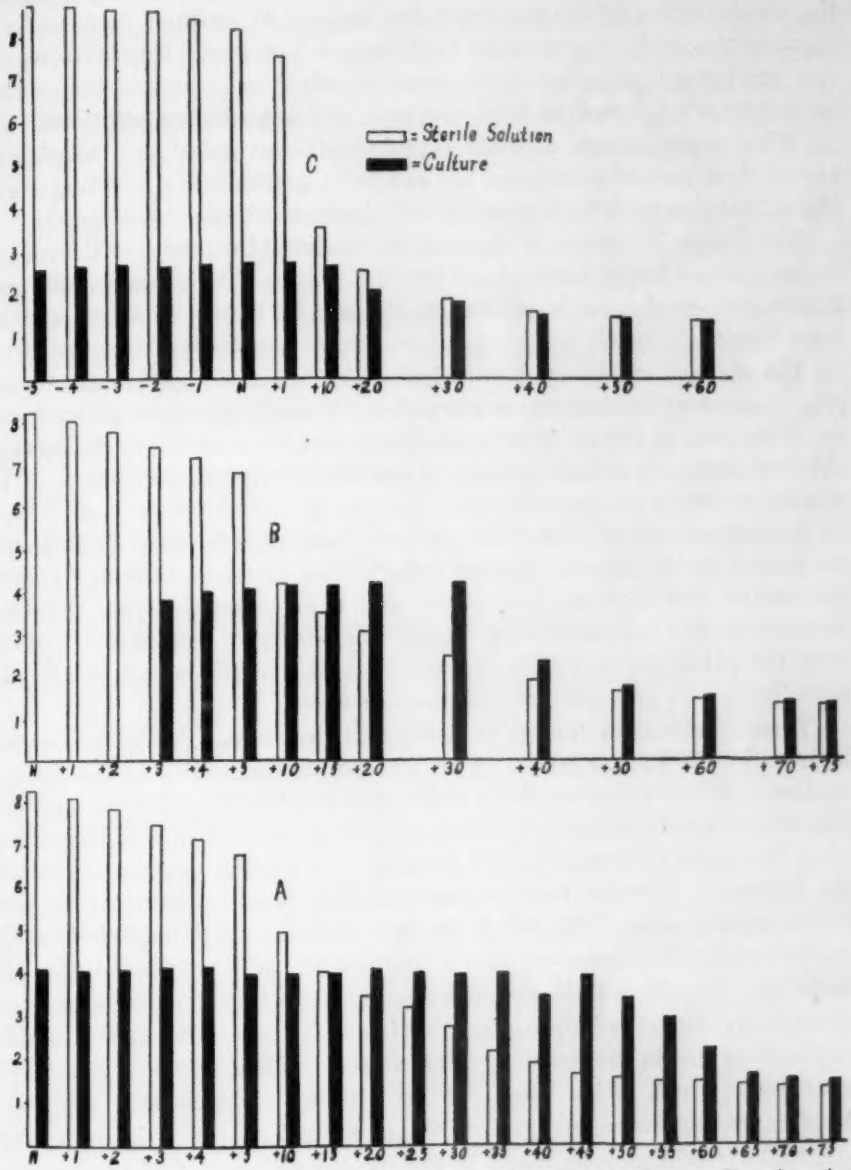

FIG. 2. Changes in reaction produced by *Sclerotium rolfsii* on media of various initial reactions. A, broth; B, broth + 1 per cent NaCl; C, broth + 2 per cent saccharose.

dently produced much faster than bases, as the acidity of all solutions was increased.

This end-point concentration in broth cultures was also independent of the concentration of the nutrients, the amount of medium used, and the depth of the liquid in proportion to the exposed surface. Furthermore, the end point was approximately the same in solutions of any of the proteid substances tried, including broth, peptone, and egg albumen solutions.

When sugar, starch, or other carbohydrate was added to a solution of any of these proteid substances, the end point concentration was very much higher, varying with the amount of carbohydrate added.

The change in reaction of the medium produced by growth of the fungus on beef-extract broth, broth plus 1 per cent sodium chloride, and broth plus 2 per cent saccharose is shown graphically in figure 2, A, B, and C, respectively.

The effect of several other carbohydrates and a few organic acids on the final reaction of broth cultures was tested. The cultures were grown on 50 cc. of medium in 100 cc. Erlenmeyer flasks, and the results are the average obtained from one culture of each of the four strains of the fungus. The results are shown in table 2.

Lactose and glycerin were the poorest of any carbohydrate media tested for growth of the fungus. In broth the lactose appeared to retard growth during the first three to four weeks, and H-ion determinations indicated that lactose was not assimilated during this period of slow growth. However, the pH at the end of six weeks indicated that the lactose was finally assimilated. In milk similar conditions occurred.

These results show further evidence that the assimilation of any carbohydrate by the fungus results in an increase in the acidity of the culture medium. When either oxalic or citric acid is added to a protein medium the acidity is reduced by the fungous growth, a result similar to that noted when inorganic hydrochloric acid is added to a protein medium. There is the difference, however, that the loss in acidity is not so great in the case of the organic acids. The results suggest that the oxalic and citric acids assimilated by the fungus are partly changed into other acids, and excreted again into the culture fluid, although negative tests for the presence of other common organic acids indicate that this is not the case. The most probable explanation lies in the much greater titrable acidity of the organic acid solutions of equal H-ion concentration with hydrochloric acid solutions. Another explanation of the greater change toward neutrality in broth plus hydrochloric acid may be found in the specific action of HCl in changing the proportions of ammonia and of oxalic acid formed from peptone. Results to support this statement are reported under "Metabolic Products."

Citric acid appears to be a very good source of carbon for growth of the fungus. Luxuriant growth was produced on pure lemon juice, and the addition of 2 per cent citric acid to a peptone solution stimulated growth very markedly. A 2 per cent solution of oxalic acid in broth prevented all growth, and even a 1 per cent solution in broth was decidedly toxic. This toxicity appears to be due entirely to free hydrogen-ions in the comparatively highly ionized oxalic acid. That this is the case is shown by comparison with broth plus hydrochloric acid at the same H-ion concentrations.

During the progress of the work it was found that the fungus grew well on a solution of pure saccharose in distilled water. It seemed desirable, therefore, to compare the amount of growth and the final H-ion concentration produced in single nutrient solutions. For this purpose, 2 per cent solutions in distilled water were prepared, flasked 50 cc. per flask, autoclaved, and inoculated with the four strains of *Sclerotium rolfsii* used in the previous experiments. The four cultures and an uninoculated check flask of each solution were incubated 31 days at 25° C. The H-ion concentration of the liquid was then determined, and tests were made for oxalic acid. The mycelium from the four cultures was collected on a weighed filter, washed thoroughly, dried, and weighed, and the weight of the whole divided by four to obtain an average. The results are shown in table 3.

TABLE 2.—*The growth of Sclerotium rolfsii and the changes in reaction produced on various nutrients*

Medium	pH of check	pH of culture	Relative production of mycelium	Relative production of sclerotia
Broth	6.9	4.0	++	++
Broth + 2% saccharose	6.5	2.5	++++	+++
Broth + 3% saccharose	6.2	2.125	+++++	+++
Broth + 2% dextrose	6.8	2.75	++++	+++
Broth + 3% dextrose	5.8	2.625	++++	+++
Broth + 3% starch	6.8	2.85	+++++	++
Broth + 2% lactose	6.3	2.6	+++	+++
Broth + 2% glycerin	6.6	2.5	+++	++
Broth + 2% oxalic acid	1.3		No growth	
Broth + 1% oxalic acid	1.4	1.55	+	+
Broth + ½% oxalic acid	2.1	3.175	+++	++
1% peptone	7.0	4.175	+	+
1% peptone + 2% mannite	7.0	2.525	++++	+++
1% peptone + 2% citric acid	2.6	3.125	++++	+++
2% egg albumen	7.0	4.1	++	+
2% egg albumen + 2% dextrose	6.4	2.1	+++++	++
Separated milk	6.8	2.95	++	+

Slight growth was produced also on 2 per cent solutions of tannic acid, succinic acid, ethyl alcohol, and esculin; but none on acetic, lactic, or oxalic acids, asparagin or brucine.

TABLE 3.—*The growth of Sclerotium rolfsii, the reaction changes produced, and the results of tests for oxalic acid on 2 per cent aqueous solutions of single nutrients*

Media	Weight mycelium mg.	pH, check solution	pH, cultures, average	Occurrence of oxalic acid
Saccharose	47.00	6.5	2.3	present
Dextrose	25.00	4.2	2.4	do
Maltose	66.50	6.4	2.4	do
Lactose	10.00	4.2	2.8	do
Mannite	13.25	7.0	6.3	trace
Amygdalin	40.00	4.0	2.4	present
Citric acid	11.25	2.2	2.2	none
Tartaric acid	7.50	2.0	2.0	do

Oxalic acid was produced in all cultures where the fungus made any appreciable growth, except citric and tartaric acid cultures. In amygdalin cultures, hydrocyanic acid was also produced. The solution had a strong odor of almond oil and a strong Prussian blue color when treated with sodium hydroxide, ferric chloride, and hydrochloric acid. The oxalic acid was probably formed in this case from the glucose of the decomposed amygdalin molecule.

COURSE OF CHANGE IN REACTION OF MEDIUM DURING GROWTH OF FUNGUS

In order to determine the rate of change in reaction of the substratum and the time necessary to reach the end-point reaction, three series were set up in sufficient numbers to allow determination of reaction in one culture daily over a long period of time. For this purpose, standard beef-extract broth was made in 6-liter lots. To one-third of the broth, dextrose was added to make a 2 per cent solution; another third received ½ per cent glucose; and the remainder was used as standard broth. All three lots were then flasked, 25 cc. per flask, autoclaved, and 60 flasks of each lot inoculated with a single sclerotium of the pepper strain. The H-ion concentration of one check flask of each lot was tested (colorimetrically) at this time and one inoculated flask tested each 24 hours during the first 12 days. After this time the change in pH was slower, and tests were made at 48-hour intervals until the sixty-first day. At the close of the experiment, check (uninoculated) flasks were again tested and showed no change in reaction.

The results are shown graphically in figure 3. At the end of the first 24 hours only slight growth from the sclerotia could be seen on broth and on broth plus ½ per cent dextrose, and no change in reaction had occurred. On broth plus 2 per cent dextrose, more growth was evident, and the medium was slightly more acid than at the beginning. After this the reaction changed rapidly toward the acid side—most rapidly in dextrose

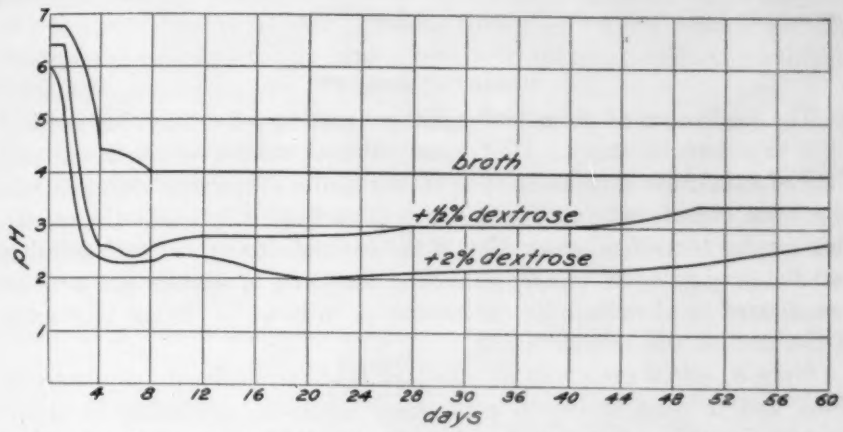

FIG. 3. Curves showing rate and direction of reaction changes produced by *Sclerotium rolfsii* on beef-extract peptone broth, broth + ½ per cent dextrose, and broth + 2 per cent dextrose.

broth—until a maximum acidity was reached. In broth this maximum or end-point, near pH 4.0, was reached on the eighth day and remained constant thereafter. In broth plus ½ per cent dextrose, the maximum acidity, pH 2.4, was reached on the sixth day. Thereafter there was a reversal, and the reaction changed slowly toward neutral once more. In broth plus 2 per cent dextrose, the maximum acidity, pH 2.0, was reached on the twentieth day, after which a reversal toward neutral occurred. In each of the latter, tests for reducing sugars at the time of maximum acidity gave negative results.

A change in the physical state of the medium began also about this time. At the time of maximum acidity most of the liquid of the medium was held in a gelatinous mass about the submerged fungous mycelium. After reversal in the reaction change, this gelatinous mass gradually disappeared, and little remained at the end of the experiment.

Whether or not the reversal in reaction was brought about by assimilation of the acids previously formed, as found by Wolff (23) to occur in cultures of *Bacterium diphtheriae*, has not been determined. At any rate

the reversal was never so complete as in broth brought to an even greater H-ion concentration by the addition of hydrochloric acid. The behavior is remarkably similar to that on broth plus ½ per cent oxalic acid in which the change appears to be due partly to natural decomposition of the acid. The fact that the loss of acidity continued after fungous growth had ceased (about the thirtieth day) is further evidence of the latter theory; though this continued change may have been in part due to liberation of ammonia previously held inside the fungous hyphae.

METABOLIC PRODUCTS

The mechanism of the reaction changes produced by fungi has received little experimental study. Most exact cultural studies of the H-ion relations of fungi have been made on synthetic media containing inorganic salts plus some organic substance as a source of carbon. Obviously the possible changes due to unequal absorption of the various elements of such solutions and the production of organic acids and ammonia by the fungus were too complicated to allow definite conclusions as to how the change in reaction of the medium was brought about.

Since *S. rolfsii* grew well on standard beef-extract broth, peptone solutions, and to some extent on pure sugar solutions, producing in each a characteristic change in reaction, it seemed possible that an insight might be gained into the nature of metabolism of the fungus by determination of the metabolic products in such simple solutions.

For this purpose a solution of 1 per cent peptone and another of 1 per cent peptone plus 2 per cent dextrose were made up in liter quantities in 2-liter flasks, autoclaved, inoculated with a single sclerotium of *S. rolfsii* from pepper, and incubated at 25° to 27° C. for six weeks. Standard broth solution plus 20 grams saccharose was made up in 500 cc. of distilled water. In another flask 30 grams of pure calcium carbonate were added to 500 cc. distilled water. After sterilization the saccharose broth was added to the calcium carbonate, inoculated and incubated at 25° to 27° C.

A 3 per cent saccharose solution in distilled water was flasked, 100 cc. per flask, and autoclaved. Eight flasks were inoculated and incubated as above. After incubation the fungous growth was removed and the liquid from the eight flasks combined for chemical examination.

All the cultures were tested for various substances by the method of Eyre (5). The results are shown in table 4.

In all media containing peptone the principal products found were ammonia and oxalic acid. Some succinic acid was present in all cultures, but the amount appeared to be small. The same was true of formic acid. The total volatile acid from 400 cc. of the saccharose culture was neutralized by

0.8 cc. N/20 NaOH. The calcium oxalate from the same solution amounted to 140 mg., equivalent to approximately 121 mg. of free oxalic acid. The presence of this trace of formic acid in all cultures without the association of any alcohol or aldehyde would indicate its origin through decomposition of oxalic acid during the two distillations.

Cultures on 1 per cent peptone solution were tested at various stages of growth for indol, skatol and tryptophane. None was indicated in any test with the para-dimethylamidobenzaldehyde-HCl test; but a positive test for tryptophane was obtained in one culture with the more delicate vanillin—H_2SO_4 test.

Several tests for the presence of urea and for glycerin in broth and in egg albumen cultures gave negative results.

When the filtrates from peptone or egg albumen cultures were slowly evaporated at 50° C., a small amount of crystalline precipitate was found; but, on account of the small amount available and the difficulties of purification, the exact physical constants of the crystalline body were not deter-

TABLE 4.—*Results of qualitative analyses of liquid media after six weeks growth of Sclerotium rolfsii*

Substances tested for	Results of tests in various media			
	Peptone	Peptone + 2 per cent dextrose	Broth + 2 per cent saccharose + 30 grams $CaCO_3$	3 per cent saccharose
Ammonia	+	+	+	0
Alcohol	0	0	0	0
Aldehyde	0	0	0	0
Acetone	0	0	0	0
Acetic acid	0	0	0	0
Formic acid	tr	tr	tr	tr
Butyric acid	0	0	0	0
Proprionic acid	0	0	0	0
Cholic acid	0	0	0	0
Glycocholic acid	0	0	0	0
Taurocholic acid	0	0	0	0
Lactic acid	0	0	0	0
Oxalic acid	+	+	+	+
Succinic acid	+	+	+	+
Benzoic acid	0	0	0	0
Hippuric acid	0	0	0	0
Salicylic acid	0	0	0	0
Tannic acid	0	0	0	0
Gallic acid	0	0	0	0

mined. Chemical tests were positive for ammonia and oxalic acid and led to the inference that it was either the neutral or acid salt of ammonium oxalate.

Since qualitative examinations of cultures indicated that ammonia and oxalic acid were formed in considerable quantity from peptone and other media, it seemed desirable to learn whether or not the quantities formed were sufficient to produce the reaction changes which occurred.

For quantitative determination of oxalic acid the various media were prepared in 1-liter quantities in 2-liter flasks, autoclaved, inoculated with a single sclerotium from the pepper strain of the fungus, and incubated 31 days at 25° to 27° C. The mycelial mat was then removed, washed several times with distilled water, placed on a watch glass, dried 24 to 48 hours in

TABLE 5.—*Amounts of oxalic acid found in 1-liter quantities of the media indicated, 31 days after inoculation with Sclerotium rolfsii*

Medium	Mycelium, mgs.	Calcium oxalate, mgs.	Equivalent oxalic acid, mgs.
Beef-extract peptone broth	676	930	802
Beef-extract peptone broth + 2% saccharose	7575	1885	1625
1% peptone	469	845	728
2% do	705	2635	2271
3% do	760	550	474
4% do	No growth		
1% do + 2% saccharose	4352	2520	2172
1% do + 4% do	7895	5105	4401
½% do + 2% do	2740	1470	1267
2% do + 2% do	5340	4645	4004
4% do + 2% do	8620	7295	6288
1% do + 2% dextrose	5675	2000	1724
1% do + 2% maltose	7505	3045	2625
1% do + 2% corn starch	6277	2600	2241
1% do + 2% mannite	1025	850	733
1% do + 2% levulose	4750	1520	1310
1% do + 2% lactose	891	620	534
1% do + 2% glycerin	1580	1000	862
1% do + 2% citric acid	1050	10	9
1% do + 2% potassium citrate	No growth		
1% do + 2% calcium citrate	No growth		
2% calcium citrate + 10 cc. N/1 HCl	955	320	276
Czapek's solution	765	345	297
Decoction from 391.5 gms. string beans	2313	2180	1879
391.5 gms. string beans after extraction	Not determined	2530	2181

an oven at 45° C. and for another 24 hours at room temperature, and then weighed.

The culture fluid and washings from the mycelium were made slightly alkaline with ammonia, a concentrated solution of calcium chloride added, and allowed to stand 24 hours at 45° C. The supernatant liquid was then decanted off, the precipitate treated with strong acetic acid, filtered, washed with distilled water, re-dissolved from the filter with hydrochloric acid and again precipitated by neutralizing with ammonia. After standing 24 hours, the precipitate was collected on weighed filter paper, again washed with strong acetic acid and several times with distilled water, air dried in the same manner as the mycelium, and weighed. The results are shown in table 5. While this method of estimating total oxalic acid is not accurate, tests with peptone solutions containing known amounts of oxalic acid indicate that the error is comparatively small. Usually there is a slight loss of calcium oxalate through the filter paper.

For determination of ammonia production, culture solutions of broth and of broth plus 2 per cent saccharose were prepared and flasked, 100 cc. per flask, autoclaved, inoculated, and incubated six weeks at 25° C. Four strains of the fungus were used in each medium and one flask of each held as a check. The fungous mycelium was then filtered off, washed with distilled water, dried as in the previous experiment, and weighed. The filtrate was made up to 100 cc. with washings from the mycelium and placed in a Kjeldahl flask. Then 50 cc. were distilled over into 100 cc. of N/20 H_2SO_4 solution. Methyl red was then added as indicator and the solution titrated to neutrality with N/20 NaOH solution. The ammonia present was estimated from the difference in amount required to neutralize the check and the culture. The results are shown in table 6.

Another series of three flasks of each medium was run as a check on the previous test. Only the pepper and potato strains of the fungus were used and the third flask of each medium held as check, all being incubated 30 days at 25° C. The averages for the two strains were: broth, 147.5 mg. mycelium; 21.9 mg. NH_4; broth + 2% saccharose, 1,000 mg. mycelium; 9.8 mg. NH_4.

There appeared to be some disintegration of the mycelium at the end of the six-weeks' period. This may have affected both the weight of mycelium and the amount of ammonia in solution, doubtless increasing the latter by an amount formerly held within the hyphae.

The most striking feature of the results is seen on the two media, broth and broth + 2 per cent saccharose. Although the mycelium produced on saccharose broth is several times as much as that produced on broth, the amount of ammonia produced is only about half that produced on the latter medium.

TABLE 6.—*Amounts of mycelium and of ammonia produced by Sclerotium rolfsii on 100 cc. quantities of broth and broth plus 2 per cent saccharose*

Strain of *Sclerotium rolfsii*	Medium	Mycelium, mgs.	NH_4, mgs.	Medium	Mycelium, mgs.	NH_4, mgs.
Pepper	Broth	20	16.9	Broth + 2% saccharose	833	lost
Potato	do	65	20.1	Broth + 2% saccharose	900	11.9
Peach	do	.....	.....	Broth + 2% saccharose	1130	12.6
Cedrus	do	150	30.7	Broth + 2% saccharose	1120	10.2
Average		78.3	22.5		995.75	11.93

Reference to table 5 shows that oxalic acid production is much greater on saccharose broth than on plain broth. This increased oxalic acid and decreased ammonia production explains the much greater acidity produced in broth plus sugar and also suggests an explanation for the formation of both ammonia and oxalic acid in broth cultures. They are both probably waste products in the utilization of certain amino acids for growth energy and carbon nutrition in production of cell walls, etc. This theory is supported by the fact that ammonia production is greatly reduced by the presence of a readily available carbohydrate, such as dextrose; and oxalic acid production is almost entirely eliminated by the presence of citric acid, a readily available source of carbon which yields no oxalic acid.

In order to determine the proportions of ammonia and oxalic acid formed in broth cultures, the mycelium was filtered from a 500 cc. broth culture which had been incubated 30 days and the filtrate made up to 500 cc. with

TABLE 7. *Actual and molecular-equivalent concentrations of ammonia and of oxalic acid in Sclerotium rolfsii culture solutions*

Culture solution	pH	NH_4, mgs. per L.	Per cent. molecular concentration	Calcium oxalate, mgs. per L.	Oxalic acid, mgs. per L.	Per cent. molecular concentration
500 cc. broth, 30-days-old	3.8	227.00	1.200	2540	2189.5	1.737
Composite broth, 2-months-old	—	394.80	2.185	3255	2805.8	2.226
500 cc. broth + HCl, 30-days-old	3.8	346.04	1.915	935	806.0	0.623

washings from the mycelium on the filter paper. Two 100-cc. lots were used for ammonia determinations, and the other 300 cc. used for the determination of oxalic acid. For comparison, three 100-cc. broth cultures two-months-old in which the mycelium had noticeably disintegrated were filtered together, and the filtrate made up to 300 cc. Of this one 100-cc. lot was used for ammonia determination and the other 200 cc. for oxalic acid determination. Also a 30-day-old culture on 500 cc. of broth plus 10 cc. N/1 hydrochloric acid was included in order to learn how the free hydrochloric acid might be eliminated by the fungous growth. Two 100-cc. portions of the filtrate were used for ammonia determination, 200 cc. for oxalic acid and the other 100 cc. for determination of chlorides in solution.

In the latter, the chlorides were determined volumetrically by the Volhard-Harvey method [see Hawk (7), p. 577]. All of the chlorine added was found in solution, showing that it is not absorbed and held in combinations inside the mycelium, a theory once suggested as an explanation of its lack of effect on the end-point reaction of broth cultures.

The results of the ammonia and oxalic acid determinations, calculated for 1-liter portions, are shown in table 7. In the filtrate from both cultures on standard beef-extract broth, ammonia and oxalic acid were found in almost equimolecular concentrations. This was especially true in the old cultures where the solutes formerly held in the fungous hyphae had presumably passed into the culture solution.

In order to compare H-ion concentrations in acid ammonium oxalate solutions in concentrations equivalent to those formed in culture solutions, M/10 solutions of ammonium oxalate and of oxalic acid were prepared, and 500 cc. of each solution poured together into a flask. The mixture was then corked and set in a warm dark place for 10 days to allow chemical equilibrium to be reached. The solution was then shaken thoroughly and portions diluted to equal concentrations with distilled water and with beef-extract broth. The pH values of each dilution (colorimetric determination) are shown in table 8.

Although the concentration of peptone and beef-extract was considerably lowered by the addition of large quantities of oxalate solution, yet it

TABLE 8.—*Hydrogen-ion concentrations in various concentrations of acid ammonium oxalate in water and in broth*

	M/20	M/50	M/100	M/200	M/500	M/1,000	M/5,000	M/10,000
pH in H_2O	3.0	3.05	3.1	3.3	3.50	3.7	4.0	4.1
pH in broth	3.4	3.90	4.3	4.5	4.55	4.6	4.7	4.8

showed considerable buffer action. The diluted solutions were probably comparable to the solution left after growth of the fungus. In broth, acid ammonium oxalate produces an H-ion concentration near pH 4.0 over a wide range of concentrations comparable with concentrations produced by the fungus in broth cultures.

Further work will be necessary to explain definitely why ammonia and oxalic acid are formed in equimolecular proportions. The most likely hypothesis is that they are both formed as waste products in the utilization of the two mon-amino di-basic acids, glutamic and aspartic acid, for carbon nutrition. In the presence of free hydrochloric acid the proportion of ammonia to oxalic acid is much higher. Possibly the free hydrochloric acid makes other amino acids more easily decomposed by the fungus. On the other hand the reduced amount of oxalic acid may be due to the rapid decomposition of free acid in solution, but in this case we must assume a stimulative effect of hydrochloric acid to account for the greater ammonia production. If the former hypothesis is accepted, we need assume no effort on the part of the fungus to regulate the H-ion concentration. The end-point reaction is produced by the waste products from the utilization of the most readily available source of carbon.

TOXICITY OF CULTURE SOLUTIONS, OF OXALIC ACID, AND OF OXALATES

For the purpose of testing the toxicity of culture solutions in which *Sclerotium rolfsii* had grown, seed of various plants were surface-sterilized and placed on the surface of sterile tap water agar (1½ per cent agar) in large test tubes. As the seed germinated, the roots pushed down into the agar and the plumule was raised sufficiently to allow a quantity of solution to be poured around the stem without coming into direct contact with the younger growing parts.

In the first test a month-old culture on 100 cc. of broth plus ½ per cent dextrose was filtered through a sterile filter into a sterile flask. The filtrate was then divided into two approximately equal portions. One portion was boiled for one minute and then allowed to cool to room temperature. The unboiled solution was poured about the base of four tomato and seven peanut plants growing in tubes as described previously. At the same time the boiled solution was poured about a similar number of seedlings. The same number received a sterile broth of the same age.

After 48 hours the stems of all the tomato plants in both boiled and unboiled culture filtrate were girdled, and the plants had collapsed. There were many small lesions on the peanut stems, but none of the plants was dead. The leaves of all plants were killed wherever touched by either of the culture filtrates. Neither tomato nor peanut plants showed any evi-

dence of injury from contact with the sterile broth. In the peanut seedlings the epidermis at the base of the stem was rather heavily cutinized and

TABLE 9.—*Toxicity of filtrates from Sclerotium rolfsii cultures on various nutrient solutions to seedlings of pepper, tomato, and soybean*

Culture filtrates	pH	Plants used	No.	No. plants injured		
				After 18 hours	After 48 hours	After 7 days
Broth	4.0	pepper	4	0	0	0
		tomato	2	0	0	0
Check – sterile broth	6.8	soybean	8	0	0	0
Broth + 2% dextrose	2.6	soybean	10	6, 1 dead	6, 1 dead	6, 1 dead
		tomato	5	5	5 dead	5 dead
		pepper	8	0	4 dead	4 dead
Broth + 2% dextrose boiled	2.8	soybean	12	4	4	6, 1 dead
		tomato	2	2	2 dead	2 dead
		pepper	5	0	3 dead	3 dead
1% peptone + 2% saccharose	2.3	soybean	4	2	3	3[a], 1 dead
		tomato	12	8	12 dead	12 dead
1% peptone + 2% saccharose, boiled	2.4	soybean	4	0	3	3[a], 1 dead
		tomato	9	6	9 dead	9 dead
1% peptone + 2% saccharose + $CaCO_3$	3.0	soybean	5	0	1	3[a], 2 dead
		tomato	11	0	3	11 dead
1% peptone + 2% lactose (30-day culture)	4.1	soybean	4	0	0	0
		tomato	14	0	0	0
2% lactose in distilled H_2O	2.7	tomato	14	0	0	3 dead
3% saccharose in distilled H_2O	2.2	soybean	4	2	.....	4
		tomato	8	5	.....	3[a], 2 dead
3% saccharose in distilled H_2O, boiled	2.2	soybean	9	4	.....	9
		tomato	5	2	.....	3[a], 5 dead
3% saccharose in distilled H_2O, sterile	6.5	soybean	2	0	0	0
		tomato	6	0	0	0
3% saccharose in tap water	2.1	soybean	3	3	3	3
		tomato	7	7	7	1[a], 6 dead
3% saccharose in tap water, boiled	2.1	soybean	4	4	4	4
		tomato	9	9	9	9 dead

[a] These plants were girdled.

contained few or no stomata. Such stems are comparatively impervious to solutions; hence peanut seedlings were not used in subsequent tests.

The results of the test indicated at once that some thermo-stable toxic substance capable of killing the tissue of tomato and peanut stems was present in the filtrate from dextrose broth culture, but was not present in sterile broth.

In order to gain some further insight as to the nature of the toxic substance, a large number of seedlings of tomato, pepper and soybean were grown in tubes of sterile tap water agar and in some cases on distilled water agar, and the toxicity of the filtrates from cultures on various media was tested. A summary of the results is shown in table 9.

The first evidence of injury from the culture solution filtrates was a bleaching of the epidermis in direct contact with the solutions, followed after a few hours by sunken lesions or complete girdling of the stems. Where the stem tissue was killed throughout, the seedling dropped down of its own weight. The plants receiving sterile culture solutions not only were not injured but after a few days started more vigorous growth than check plants. This was especially noticeable in tubes receiving sugar solutions.

No noticeable injury was produced by any culture solution less acid than pH 3.9, and the severity of the injury appeared to increase directly with increase in acidity. The toxicity is probably due solely to the hydrogen ions. The filtrate from a culture on cane sugar in water was very toxic although it contained less than one-fifth as much oxalic acid as the non-toxic filtrate from broth cultures.

Preliminary tests with solutions of oxalic acid showed this acid to be very toxic, producing lesions on the stems of seedlings indistinguishable from those produced by filtrates from S. rolfsii cultures (Fig. 4). In order to determine whether or not it would be injurious in solutions comparable with the filtrate from a pure sugar solution culture, a series of very dilute solutions were tested on seedlings. All solutions had produced some injury at the end of 24 hours. In 1–10,000 solutions the injury was very slight but was more evident at the end of 48 hours. At the same time a 1 per cent solution of ammonium oxalate had produced no injury. By the end of 48 hours, however, molds had begun to develop on some of the solutions, and in further tests records were taken during the first 24 hours only.

As cutinized tissues are not readily penetrated by solutions and do not show slight injuries readily, the toxicity of oxalic acid, ammonium oxalate and urea oxalate was compared on disks of red beet root tissue. A longitudinal cylinder, 1.5 centimeters in diameter, was cut from a beet root with a sharp cork borer and was then cut into disks one-half to one millimeter

FIG. 4. Soybean seedlings from agar tube cultures, showing stem tissue bleached and collapsed after 72 hours contact with toxic solutions: (a) filtrate from 13-day-old culture of *S. rolfsii* on broth plus 3 per cent saccharose, (b) 0.5 per cent solution of oxalic acid in sterile broth, (c) 0.1 per cent solution of oxalic acid in distilled water. Natural size.

thick. The disks were washed several times in distilled water and distributed five to each solution in culture dishes containing 30 cc. of solution. In non-toxic, dilute solutions the disks of beet root tissue became more turgid

FIG. 5. Pepper stem showing mycelium of *S. rolfsii* on surface (natural infection).

than at the beginning and retained the red pigment. In lethal solutions the pigment diffused into the liquid and the disks became flabby, as could

TABLE 10.—*Toxicity to plant stems and tissues of oxalic acid, ammonium oxalate, and urea oxalate as indicated at the end of 24 hours*

Oxalic Acid

Plants	No. in each solution	Concentrations			
		1–1000	1–2000	1–5000	1–10,000
Tomato	6	dead	dead	injured	uninjured
Pepper	2	do	girdled	girdled	do
Cotton	6	do	dead	dead	injured
Beet root disks	5	do	do	do	partly bleached

Ammonium Oxalate

Plants	No. in each solution	Concentrations					
		3–100	2–100	1–100	1–200	1–500	1–1000
Tomato	6	.....	.....	uninjured	uninjured	uninjured	uninjured
Beet root disks	5	uninjured	uninjured	do	do	do	do

Urea Oxalate

Plants	No. in each solution	Concentrations					
		1–200	1–500	1–1000	1–2000	1–5000	1–10,000
Tomato	6	dead	dead	injured	uninjured	uninjured partly bleached	uninjured
Beet root disks	5	do	do	dead	dead		do

TABLE 11.—*Toxicity of acid ammonium oxalate to beet root disks*

Solvent	Concentrations						
	M/20	M/50	M/100	M/200	M/500	M/1000	M/10,000
H_2O	dead	dead	dead	partly dead	slightly bleached	uninjured	uninjured
Beef-extract broth	do	do	slight injury	uninjured	uninjured	do	do

be seen when they were lifted by one edge. Near the limit of lethal dilution the thinner portions of the disks were bleached and dead, although cells in the thick portions remained alive. The results at the end of 24 hours are shown in table 10.

Because of the difficulty of obtaining the crystallized salt of mon-ammonium oxalate free of oxalic acid and of the double salt, the equi-

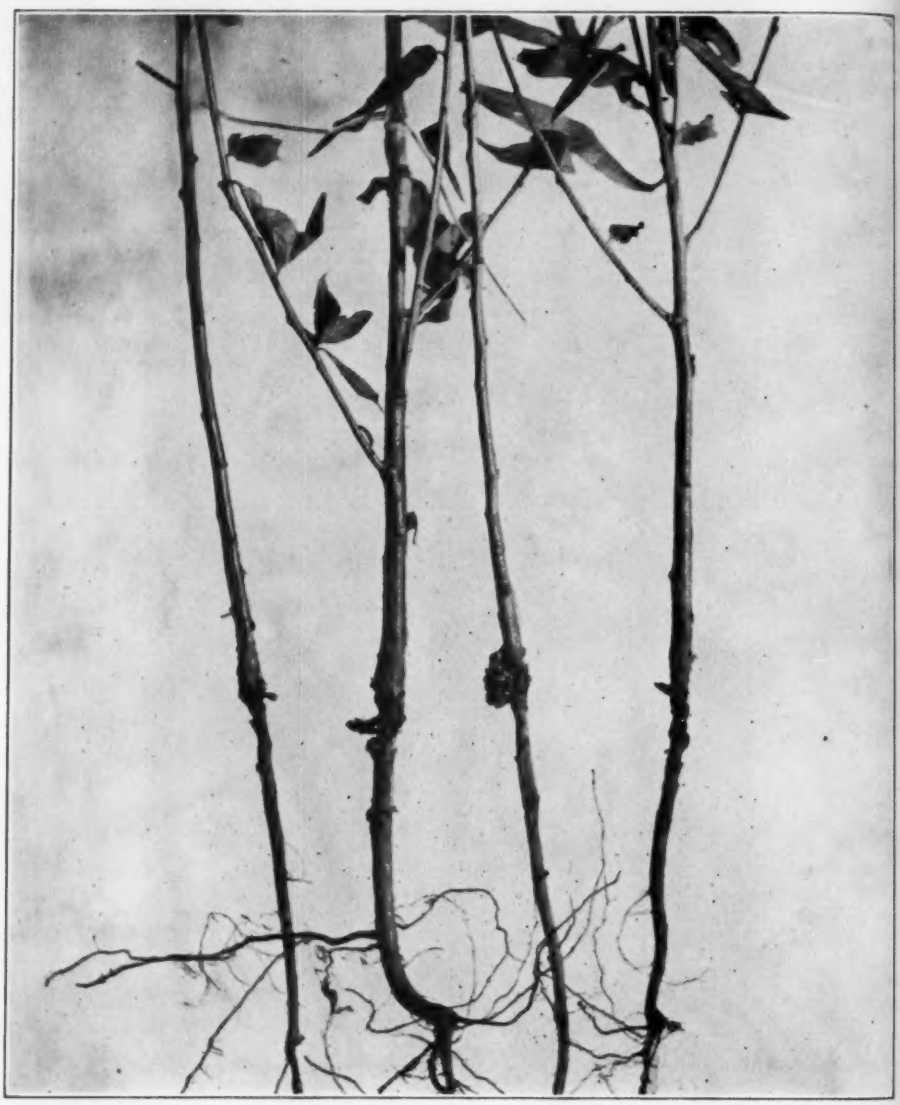

FIG. 6. Peach seedlings girdled by *S. rolfsii*.

molecular mixture of the acid and the double salt previously mentioned was used in toxicity tests. The results of the test are shown in table 11.

Comparison of the water solution with the results in table 10 shows that the acid ammonium oxalate is about equally toxic with urea oxalate and approximately half as toxic as pure oxalic acid. In broth the toxicity is greatly reduced, corresponding with the reduction in ionization in this solvent. This indicates again that toxicity is due to the hydrogen-ions in solution. This is in accord with the conclusions of Kahlenberg and True

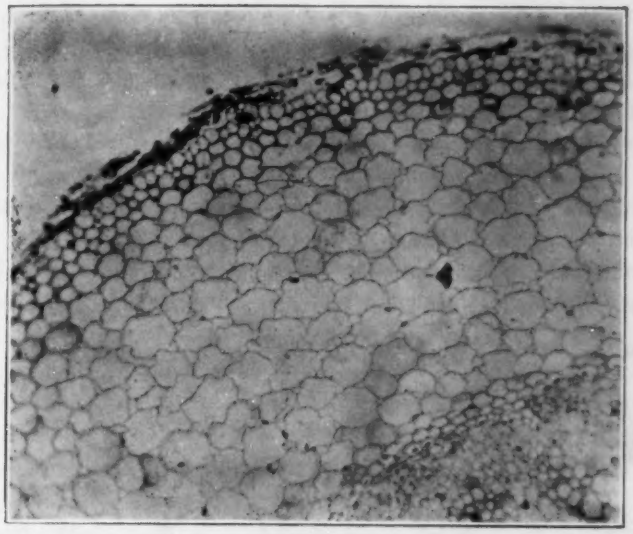

FIG. 7. Photomicrograph, portion of cross section of soybean stem in early stage of infection by *S. rolfsii*, and showing mycelium with holdfasts clinging to surface.

(8) in their study of the toxicity of oxalic and other organic acids to the roots of *Lupinus albus*, of Muth (12) in regard to the leaves and flowers of grapes and other seed plants, and of Uppal (18) concerning the spores of *Phytophthora colocasiae*. Oscar Loew (10), however, working principally with algae and also reviewing the work of Migula (11) on algae, reached the conclusion that the oxalic radical in solutions of oxalic acid and of oxalates has specific toxicity due to the precipitation of calcium from organic combinations in the nucleus.

Doubtless there is considerable variation between the various groups of plants in regard to the use of calcium and in regard to the toxic action of oxalic acid and of oxalates; but in the case of fungi and the seed plants so far tested the toxicity appears to be due to the hydrogen-ions in solution.

RELATION OF SCLEROTIUM ROLFSII TO HOST TISSUE

In the field, plants killed by *Sclerotium rolfsii* are characterized by a considerable mat of mycelium over the surface of the stem near the surface of the soil (Figs. 5 and 6). Underneath this mycelium the bark is dead and decayed, but the central cylinder of mature plants is not decayed and such plants remain erect after death.

In order to study the relation of the mycelium to the host tissues, tomato and soybean seedlings were grown in tubes of sterile tap water agar. After the seedlings were well started, a single fungous sclerotium was dropped onto the surface of the agar at some distance from the seedling. A thin cobwebby growth of mycelium was produced over the surface of the agar. When it came into contact with the stem of the seedling, a thick white mat was formed over the base of the stem, but the plant was not killed until two to three days later. The stems thus attacked were examined microscopically by peeling off the epidermis and mounting in water. Some of the stems in various stages of attack were killed in chrom-acetic acid, embedded in paraffin, sectioned, and stained with haematoxylin.

The fungous mycelium clings to the stems by means of peculiar holdfasts, thickened ends of hyphae which are more or less flattened on the

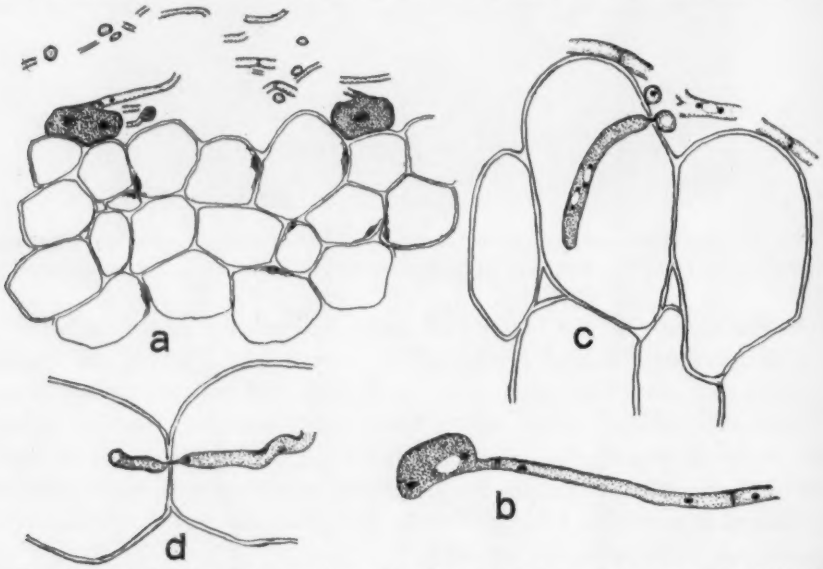

FIG. 8. (a) Section from surface of soybean stem in early stage of infection by *S. rolfsii*, showing relation of holdfasts and hyphae to epidermal cells. Both epidermal and parenchyma cells shown are already dead. (b) A single holdfast disengaged and more highly magnified. (c) Penetration of epidermal cell by hyphal branch. (d) Penetration of parenchyma cell wall by hypha.

Drawn with aid of camera-lucida. a, × 525; b, c, and d, × 1750.

surface in contact with the epidermal cells of the host (Figs. 7 and 8, a and b). The epidermal cells and the underlying parenchyma to a depth of two to four cells are killed before there is any evidence of hyphal penetration. Later the hyphae penetrate the dead cells and spread throughout the soft parenchymatous tissue. When passing through the cell walls the hyphae are much constricted and pass through very small openings (Fig. 8, c). This passage appears to be produced mechanically. In the initial penetration into the epidermal cells the outer wall is often crushed. Also there is little evidence of the dissolving action of enzymes even on the walls of parenchyma. Penetration of lignified tissues is very slow.

The epidermis with attached fungous hyphae was stripped from the base of some of the stems in early stages of infection and tested for the presence of oxalic acid by mounting in a weak calcium chloride solution, by Patschovsky's (13) ferrous-sulphate-acetic acid method and by Plahl's (15) silver nitrate-nitric acid method. All three gave crystals of oxalates in the epidermal cells, in the fungous hyphae, and on the surface of the hyphae.

In another experiment, cured sweet potato roots were washed and placed in a moist chamber. After several sprouts appeared, sclerotia from the pepper strain of the fungus were placed on the surface of the root. The hyphae spread over the root and attacked the stems of the very young sprouts directly, just as they did on tomato and soybean seedlings. On older sprouts, however, not even the mycelial mat over the surface was formed. The hyphae in some cases climbed over the surface of these older sprouts until they came into contact with a young root or leaf, which they entered and killed, and then killed the stem of the sprout.

DISCUSSION

There is nothing especially unique in the temperature relations of *S. rolfsii* so far as the minimum and maximum temperatures for growth are concerned. The fact that vegetative growth is killed by even slight freezing may be of some significance in limiting the region in which the fungus can survive the winter. The dormant sclerotia can survive at least $-10°$ C. for a short time, but sclerotia which have started growth are killed as readily as the mycelium. The very high optimum growth temperature, 30° to 35° C., and the fact that the fungus grows very poorly at temperatures below 20° C. are of greater significance. The latter is near the mean summer temperature in the cooler sections of the country. When considered in conjunction with other data reported in the present article, it leads to the conclusion that temperature relation is the principal factor in limiting the geographical range of the fungus.

The relation of the fungus to the reaction of the substratum can have little significance in relation to its distribution. Few of our arable soils are sufficiently alkaline to prevent growth of the fungus, especially where undecayed organic matter is present in the soil. In field experiments which may be reported in a later paper, great difficulty has been found in maintaining a reaction above pH 8.0 throughout a single season. In one test, where the soil had received a heavy application of cotton seed meal, hydrated lime was applied at various rates up to five tons per acre; yet at the end of four months the reaction of the plats receiving the heaviest applications of lime was barely above pH 7.0 and *S. rolfsii* was vigorously attacking plants in these plats. It seems, therefore, that maintaining an alkaline reaction of the soil as a means of protecting plants from attacks of this fungus is hardly feasible. Other methods of control are now being investigated.

Apparently, the principal function of sclerotia is to tide over periods of low food supply and unfavorable temperatures, and the length of the period of dormancy is governed entirely by these two factors. If transferred to suitable nutrients and favorable temperature at any period of development they begin to grow immediately. They have been observed to germinate and renew growth in old cultures in which the mycelium had largely disintegrated. Extracts of mycelium and even autoclaved cultures make very good media for growth, all of which indicates that dormancy is not induced by the presence of any toxic substance. The influence of a suitable nutrient on germination of sclerotia is further indicated by their failure to germinate on tap water and on water containing many organic substances not toxic to the fungus. We must infer that such substances are not sufficient to stimulate germination.

Ammonia and oxalic acid in the quantities ordinarily found in cultures are not toxic to the fungus.

The fact that certain fungi and bacteria are capable of producing oxalic acid has been known to botanists for more than 50 years. The early work and observations on the subject are summarized in several publications of Wehmer (19, 20 and 21), who made the earliest comprehensive investigations on the production of this acid by fungi growing on various classes of nutrients. Discussions of the results of subsequent investigators may be found in the more recent publications of Wehmer (22), of Currie and Thom (4), of Raistrick and Clark (16), of Gotoh (6) and of Cooley (3).

Knowing the toxic nature of oxalic acid, several botanists have suspected its responsibility for the death of host tissue previous to or during its penetration by certain species of parasitic fungi.

Many references to the work of De Bary on *Sclerotinia libertiana* Fckl. attribute to him the conclusion that this fungus produces an enzyme which kills the host tissue. [See Klebahn (9), p. 399, also Cooley (3), p. 293.]

With this conclusion in mind, the observation that when milk or egg albumen solutions were inoculated with *S. rolfsii*, the proteids were first coagulated and then slowly digested during growth of the fungus, immediately suggested that a protein coagulating enzyme was produced. Several attempts were made to demonstrate the presence of such an enzyme in culture solutions and in the mycelium but always with negative results, and the conclusion was finally reached that the coagulation of these substances in cultures was due entirely to acidity.

Upon close study of De Bary's (2) original publication, it appears that his final conclusion on the subject is indicated in the statement (p. 420) that an enzyme destroys the cell walls and may or may not be responsible for the death of the protoplasts, as organic acids (such as oxalic) and their salts are known to be toxic to plant protoplasts.

Smith (17), in his study of the parasitism of *Botrytis cinerea* on lettuce plants, found that the cells were killed, before penetration of the fungous hyphae, by a thermostable toxic substance, and expressed the opinion that this toxic substance was oxalic acid.

Peltier (14), studying the parasitism (supposedly) of the same fungus on lettuce and pepper plants, found a thermostable toxic substance in extracts from the fungous mycelium but failed to find oxalic acid in the extract. He expressed the opinion that this toxic substance was some organic acid but not oxalic.

Cooley (3) found that *Sclerotinia cinerea* produced considerable quantities of oxalic acid from plum and peach juice and from peach fruits but expressed no definite opinion as to the significance of the results.

Whatever the case may be with regard to other fungi, the evidence seems quite conclusive that oxalic acid is responsible for the death of the host tissue in the plants attacked by *Sclerotium rolfsii*. (1) Plants were killed by the filtrate from cultures on pure sugar solutions where oxalic acid appears to be the only metabolic product formed in any significant quantity. (2) In the filtrate from cultures on other media the toxicity increased directly with the increase in free acid. (3) The injuries were similar to injuries produced by pure oxalic acid solutions. (4) Considerable quantities of oxalic acid or soluble oxalates were found in the dead host cells, even before entrance of the fungous hyphae; whereas none was found in healthy cells of the same plants.

Accepting this conclusion, we can easily understand the very wide range of host species attacked by this fungus. Apparently, any plant with an epidermis easily permeable to oxalic acid solutions is susceptible to attack. The fungus does not penetrate living tissues, and there is no occasion for the development of special adaptations between parasite and host.

SUMMARY AND CONCLUSIONS

1. The minimum temperature for growth of *Sclerotium rolfsii* is near 8° C. The absolute maximum is near 40° C., but 37° C. appears to be the maximum for continuous normal growth. The optimum growth temperature lies between 30° and 35° C. Growth is poor below 20° C.

2. Temperature relations appear to be the limiting factor in determining the geographical range of the fungus.

3. The fungus does not appear to utilize inorganic nitrogen readily when supplied either as nitrates or as ammonium salts.

4. There is a slight variation in cultures of the fungus from different sources in their relation to the reaction of the substratum; but on beef-extract-peptone broth none of the cultures grew when the reaction was more alkaline than pH 8.3 or more acid than pH 1.4.

5. The addition of 1 per cent NaCl to broth shortened the growth range of the fungus on both the alkaline and the acid side of neutrality.

6. The addition of 2 per cent saccharose to broth extended the growth range on the alkaline side but shortened it on the acid side.

7. On broth and broth plus NaCl, the fungus, during growth, changes the reaction of the medium toward a fairly definite end-point near pH 4.0.

8. On broth plus cane sugar the reaction change is always toward the acid side until the sugar is used up.

9. This reaction change is due to the production of ammonia and oxalic acid from peptone and of oxalic acid from saccharose.

10. In broth cultures ammonia and oxalic acid accumulate in nearly equimolecular proportions. Apparently both are waste products in the utilization of certain amino acids as a source of energy and carbohydrate nourishment.

11. Oxalic acid is also produced from a number of other carbohydrates and from various plant decoctions. None is produced from citric or tartaric acids.

12. Filtrates from cultures of the fungus growing on certain nutrients are toxic, both before and after boiling, to seedlings of tomato, pepper and soybean.

13. The injuries produced by the filtrates are very similar to those produced by oxalic acid solutions.

14. The toxicity increases with the increase of H-ion concentration and the increase in concentration of free oxalic acid in the filtrate.

15. The toxicity of the filtrates and of oxalic acid solutions to the plants tested appears to be due entirely to the hydrogen-ions in solution.

16. In parasitic attacks on plants the fungus forms a considerable mat over the attacked portion, clinging to the epidermis by means of holdfasts.

The underlying cells are killed before the fungous hyphae enter the host tissue.

17. Considerable quantities of oxalic acid or soluble oxalates are found in these dead cells, while none is found in healthy cells of the same plants.

18. The evidence appears to be conclusive that the death of these cells is due to the toxic action of oxalic acid and that the oxalic acid is secreted by the fungous hyphae.

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STUDIES ON THE SCUTELLUM ROT DISEASE OF CORN[1]

BENJAMIN KOEHLER

INTRODUCTION

Scutellum rot is a very common disease of dent corn (*Zea mays indentata*). It also occurs in *Zea mays saccharata, Zea mays amylacea, Zea mays indurata,* and perhaps in other subspecies.

Corn kernels affected with this disease were illustrated by Hoffer and Holbert (6) in 1918, but the disease was not clearly defined or distinguished from other seedling rot diseases. Adams and Russell (1) clearly described scutellum rot as caused by *Rhizopus nigricans.* Holbert *et al.* (8) published extensive data on the field performance of plants grown from seed susceptible to this disease and named the malady "scutellum rot."

To judge from results of tests of seed corn for germination and freedom from disease conducted by several commercial seed-corn testing establishments, the disease is very common in all parts of the corn belt from which corn had been obtained. In germinating the corn exhibited in the Fifth Utility Corn Show held at Urbana, Illinois, 1925, records were made of all the corn diseases that appeared on the germinator. It was found by Koehler and Pettinger (10) that about 21 per cent of the kernels from the ear corn exhibited in the show developed a scutellum rot on the germinator. This show corn should represent, however, the best seed corn of the state. Most of the seed corn would doubtless have shown a higher percentage of infection. Many lots of seed corn obtained from the central portion of the corn belt and tested by the writer during the past six years were very nearly 100 per cent susceptible to this disease when tested on the limestone-sawdust germinator. This germinator has been described by Holbert *et al.* (8). Seed corn obtained from the Philippine Islands and from Argentina, South America, also developed this disease on the germinator.

Control measures for this and other seed-borne corn diseases have been described by Hoffer and Holbert (6), Holbert and Hoffer (7), and Holbert *et al.* (8). The recommendations consist essentially of selecting ears from sturdy, healthy stalks in the field, proper curing, culling down to a type of ear that has been found to be most disease resistant, then testing the selected

[1] Contribution from the Department of Plant Pathology, University of Wisconsin, and the Department of Agronomy, University of Illinois. Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, University of Wisconsin.

ears on a good germinator, and discarding any that show the presence of disease.

NATURE OF THE DISEASE

Typical scutellum rot usually is not caused by a parasite carried within the corn kernel, but is caused by infection from without after the kernel begins to germinate and the seed coat has been ruptured. When germinated on a limestone-sawdust germinator at about 27° C. (80° F.), most of the radicles break through the pericarp during the second day, but the plumules appear a little later, usually not until the third day. Following the rupture of the pericarp of susceptible seed, certain molds are able to enter and cause scutellum rot. On the sixth or seventh day at this temperature the rots can be seen very easily when the kernels are bisected. Kernels that fail to sprout seldom develop scutellum rot of the type herein described.

Four rows of sprouted kernels representing four different ears are shown in figure 1. The first and third rows are resistant to scutellum rot; the other rows show Rhizopus infection in abundance. A prolific growth of *Rhizopus* on a kernel is a fair indication of scutellum rot. To make a more accurate observation, however, the kernels must be bisected and examined closely. Figure 2 shows longitudinal sections of a good kernel (A), and kernels having various types of scutellum rot (B–F). In B the epithelium of the scutellum has rotted and appears dark in the illustration. C is a little more advanced stage of the same type, while D is still further advanced, the rot involving all of the cortical tissue of the scutellum. Rots of the above forms are typical for infections by species of *Rhizopus*. These organisms also ramify throughout the endosperm but do not cause a discoloration of this tissue.

Another type of rot sometimes produced by *Rhizopus*, but perhaps more often by other organisms, is shown in figure 2, E, the points of infection being at the bases of the lateral roots. When this type of infection develops early, some of the root initials may be destroyed, hence those particular roots fail to develop.

A rot at the base of the scutellum, as shown in figure 2, F, is characteristic for Aspergillus infection but may also be caused by some other organisms. Entrance of the organism apparently was effected through the tip end of the kernel and was not dependent on the rupture of the pericarp. It has been shown by Manns and Adams (14) that the dormant mycelium of *Aspergillus* spp. may be found beneath the cap at the tip of the kernels of dry corn. The writer has often found this type of rot after inoculating disease-free corn with species of *Aspergillus*. These latter organisms may, however, also cause the epithelial rots shown in B and C and the central rot

shown in E. From the condition shown in B, C, E, and F, the rot may extend to include the whole scutellum, as shown in D.

Scutellum rot infection usually results in a weakened condition of the seedlings from which the plants never fully recover. Two factors evidently cause this weakening. In the first place, the fungus destroys much of the enzyme secreting and absorbing tissue of the scutellum and separates the latter from its nutrient base. Sometimes the injury is limited to this region. In a more progressive form of the disease, the fungus enters and disorganizes the cortical tissue of the scutellum, and from there it invades the vascular tissue of the embryo. *Rhizopus* and *Aspergillus* have fre-

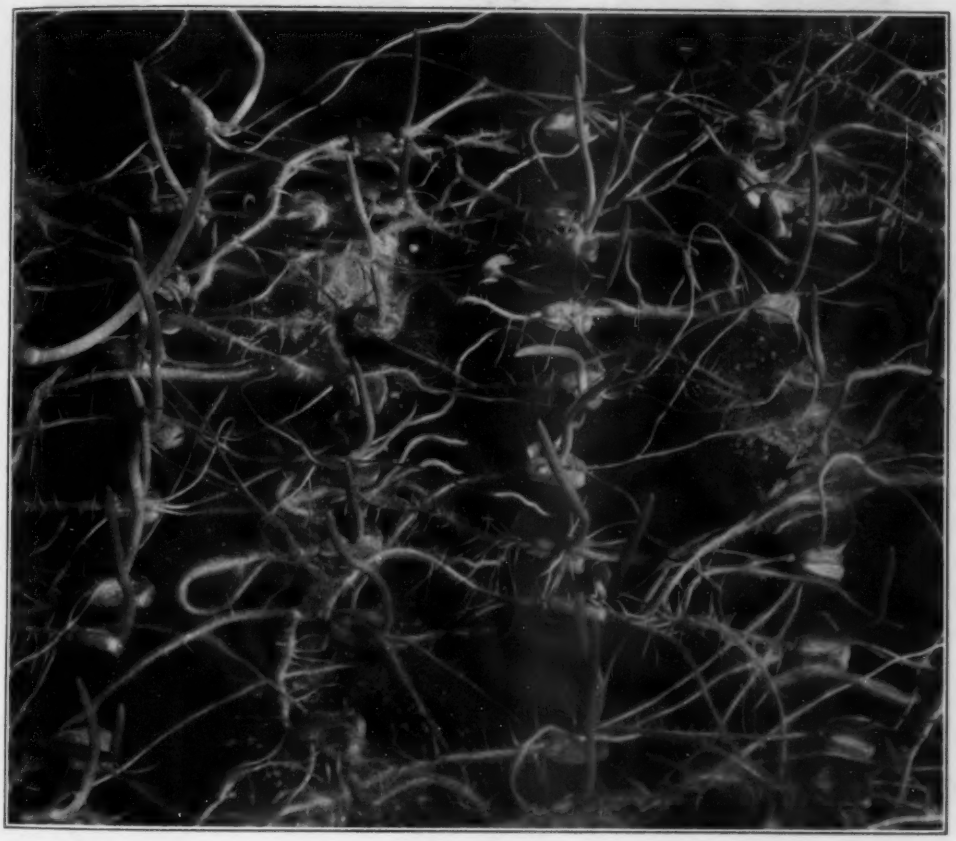

FIG. 1. Scutellum rot symptoms on the germinator. Each row of kernels represents an ear of corn. The first and third rows are free from mold. Considerable Rhizopus growth occurs in connection with the kernels of the other rows. The scutellum of such kernels usually is in a rotted condition.

quently been isolated from the mesocotyls of infected plants grown in soil, following seed inoculation with these organisms.

When seed susceptible to the disease is planted in experimental plots and its performance is compared with that of plants from disease-resistant seed, some of the features noticed are a reduced stand, weaker plants, and decreased yield. Data on 54 experiments given by Holbert *et al.* (8) over a period of seven years in different parts of Illinois show an average reduction in yield of 12.4 ± 0.66 bushels, or 17 per cent.

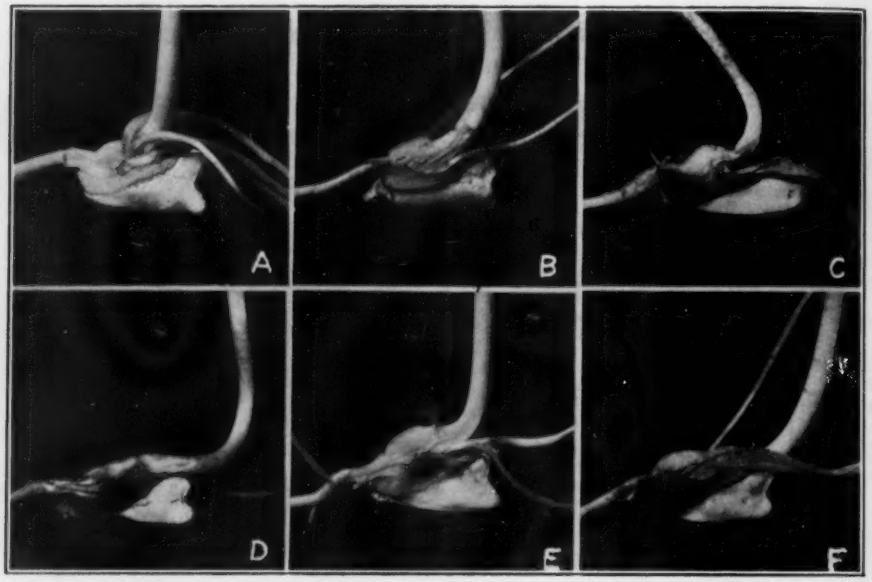

FIG. 2. Bisected kernels of germinated corn showing scutellum rot symptoms. A—healthy; B—epithelial rot of the scutellum; C—more advanced stage in which the rot has progressed to the cortical and vascular tissues; D—still farther advanced stage involving the whole scutellum; E—a rot at the base of the lateral roots; F—a rot of the base of the scutellum.

ORGANISMS RESPONSIBLE FOR SCUTELLUM ROT

Hoffer and Holbert (6) state that species of *Gibberella, Fusarium, Verticillium, Rhizopus,* and *Pseudomonas* cause diseases of corn plants, and in the same publication they illustrate typical cases of scutellum rot (Figs. 15–16). However, these authors do not mention which organisms are particularly responsible for the scutellum rot condition. Adams and Russell (1) found that *Rhizopus nigricans* is able to cause scutellum rot of corn, and they gave the pathological histology of infected kernels. Taubenhaus (15) found that the black and yellow molds of corn (*Aspergillus niger* and

A. flavus) had a detrimental effect on corn seedlings. However, he mentions only root rot as being due to these organisms and not scutellum rot. Manns and Adams (14) found that species of *Penicillium* and *Aspergillus* were limiting factors in germination. Holbert *et al.* (8) state that *Rhizopus* spp. are the most common molds causing scutellum rot on the germinator. They state further that corn subject to *Rhizopus* attack on the germinator is more susceptible to attacks of soil fungi and to injury from inoculation with other organisms than is corn that is resistant to scutellum rot on the germinator.

Pure Culture Inoculations

In order to determine whether *Rhizopus* species other than *nigricans* may cause scutellum rot, a number of species were secured for inoculation purposes. As some of these species are common in both soil and air, the tests had to be conducted under aseptic conditions. This necessitated the use of containers from which all living organisms could be excluded except those which were to take part in the experiment. A few other fungi which sometimes seem to cause scutellum rot were also included in these tests. It was shown by Lauritzen and Harter (11, 12) that different species of *Rhizopus* may behave in dissimilar manner at different temperatures. Therefore the tests were conducted under three different temperature conditions, namely, 16°, 22°, and 30° C., the first being near that of the soil temperature at corn planting time, the second representing ordinary room temperature, and the last being as high as that at which corn germinators are often run.

Source of Cultures.—Experiments were conducted with 12 species of *Rhizopus* which had been obtained from two sources. Cultures of *R. chinensis* Saito, *R. arrhizus* Fischer, *R. reflexus* Bainier, plus and minus strains, *R. microsporus* van Tiegh., plus and minus strains, and *R. pyriformis* marked "New sp. Eddy" were obtained from Dr. C. Dreschler. Cultures of *R. nodosus* Namysl., *R. nigricans* Ehrenb., *R. tritici* Saito, *R. maydis* Bruderl., *R. artocarpi* Racib., *R. oryzae* Went. and Pr. Geerlings, and *R. delemar* (Boid) Wehmer and Hanzawa were obtained through Dr. J. G. Dickson from Dr. L. L. Harter.

In addition to the above, a culture of *R. nodosus* marked "b," and cultures of *Aspergillus niger, A. flavus,* and *Penicillium* sp. were isolated from germinating corn kernels and pure lined for inoculation purposes by the writer.

Methods.—Inoculations were made on kernels from 10 ears of corn which, in a previous test on a limestone-sawdust germinator, had shown 60 to 80 per cent scutellum rot infection when inoculated with a spore suspension of *Rhizopus nodosus.* Culture dishes 100 mm. wide by 45 mm. deep were used in these tests. In preliminary trials some dishes were prepared with

soil, some with potato dextrose agar, and some with plain non-nutrient agar. When soil was used, it had to be sterilized on three successive days at 15 pounds pressure to insure sterile plates. When agar was used, one sterilization was sufficient.

In the preliminary trials in which the corn kernels were placed on sterile soil, nutrient agar, and plain water agar, respectively, and inoculated with *Rhizopus nodosus,* the resulting percentages of scutellum rot obtained were very similar. In the case of the soil and nutrient agar, a little of the mycelium with the accompanying spores was transferred to the center of the culture dish, but when plain water agar was used, it had to be placed directly on each kernel. This made the latter method a much more tedious one, with increased opportunity for contamination. On the nutrient agar the excessive fungous growth was objectionable. The tests made on soil were very satisfactory, although repeated sterilizations were necessary, excessive glass breakage occurred, and the soil etched the glass very badly during the sterilization process. A very satisfactory culture medium was finally prepared by using 1.5 per cent agar, and 1.0 per cent cornmeal. This was cooked in a steamer for one hour and then strained through fine cheese cloth. Inoculations were made in the center of the dish only, and the resulting fungous growth was not excessive but was comparable to that on soil.

Ten kernels of corn, one kernel from each of the 10 ears, were sterilized, washed, and placed in a circle, germ side up, in each dish. At the 16° temperature the corn was grown in the culture dishes for 28 days, at 22° C. for 10 days, and at 30° C. for 8 days.

Griffin low form beakers of 100 cc. capacity were used as containers in sterilizing and washing the corn. These were covered with aluminum dishes 65 mm. in diameter and 15 mm. deep.[2] (See figure 3.) One set of dry beakers with covers was sterilized by dry heat. A second set, comprising three times the number used in the first, was filled two-thirds full of water and autoclaved. The corn was introduced into the dry beakers after they had cooled. A 50 per cent alcohol solution was poured on the corn and then immediately drained off, and the beaker nearly filled with a 0.5 per

[2] These dishes are regular stock sold by dealers in scientific apparatus (Central Scientific Company, Catalog No. 3850). The Pyrex catalog quotes this size Griffin beaker only with the lip, but the manufacturers of Pyrex ware state that they will furnish them without lips if so specified in the order. These beakers do not corrode or break in the sterilizing process and withstand considerable rough handling. The Griffin low form is superior to the regular tall form beakers because they stand more securely, are easier to reach into with the forceps, and fit better with the aluminum covers. At first the tops and bottoms of 75 mm. petri dishes were used for covers, but the breakage on these in the autoclave was very high, especially when the vessels were stacked several deep. Furthermore, they were a little too large for a good fit on the beakers. The aluminum covers are very nearly the right size.

cent solution of Uspulun. The corn kernels were treated in this solution for one and one-half hours at 30° C. The above disinfectant was used in preference to the usual mercury bichloride when it was desired to germinate the corn. Uspulun, as well as some other compounds of similar nature, has very little, if any, detrimental effect on the vitality of corn kernels. When it was desired to surface-sterilize germinated kernels preparatory to making

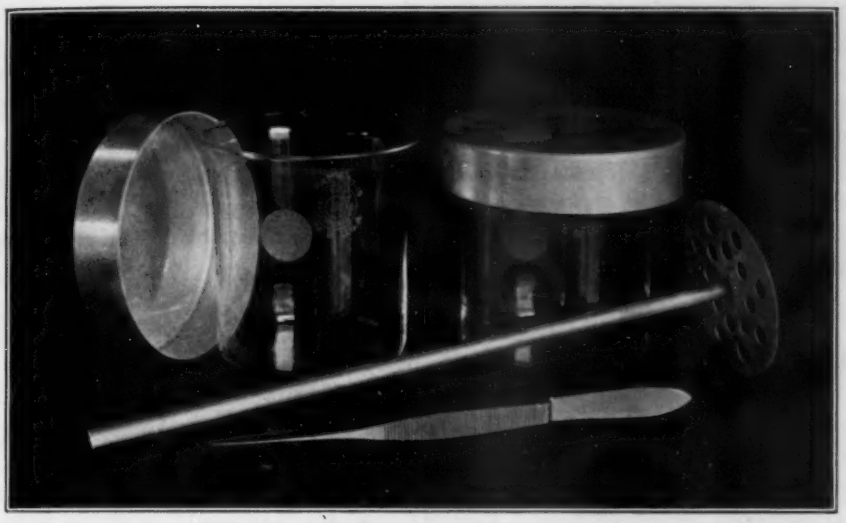

FIG. 3. Apparatus used in surface sterilizing and washing plant tissue.

fungous isolations, a five-minute treatment with mercuric chloride 1:1000 was used. After treating the corn, the solution was poured off and the kernels poured into one of the beakers prepared with sterile water. This is a more rapid operation than picking up the corn kernels one at a time with forceps. After leaving the corn in the sterile water for 20 minutes, the operation was repeated except that this time the sterile water was poured onto the corn instead of dropping the corn into the sterile water. Three washings of 20 minutes each were given. For the last two washings, if desired, the sterile water could perhaps just as well be prepared in larger flasks plugged with cotton.

In order to keep the corn kernels from leaving the beakers when the liquids were poured off, a disk of perforated zinc was cut so as to pass easily into the beakers. A metal handle was fastened at right angles to the center of the disk (Fig. 3). This retainer was kept in a beaker of 95 per cent alcohol and was flamed before using.

All the transfers were made in a special transfer room which was kept as clean as possible. Practically no trouble with contamination was experienced.

Inoculations in Culture Dishes.—Data from inoculation trials with all the Rhizopus species, as well as with several other organisms, are given in table 1. As given in this table, rot type 1 refers to the epithelial rot illustrated in figure 2, B and C, and also to the more advanced stage of this rot progressing into the cortical tissue shown in D. Type 2 refers to the central rot at the base of the lateral roots shown in figure 2, E; type 3 refers to the rot at the tip end of the kernel (base of scutellum) shown in F. The Rhizopus species are arranged in descending order according to their effectiveness in producing scutellum rot. All the species used were more or less active in this respect. Five trials of ten kernels each were made with each species at the different temperatures. Several controls were included in each series and these always remained free from infection. Very

TABLE 1.—*Percentages of scutellum rot at temperatures of 16, 22, and 30° C. when corn was inoculated with species of Rhizopus, Aspergillus, and Penicillium*

Organisms	16° C. (28 days)			22° C. (10 days)			30° C. (8 days)			Total rot in per cent			
	Type[a] and percentage of rot			Type and percentage of rot			Type and percentage of rot						
	1	2	3	1	2	3	1	2	3	16°C.	22° C.	30° C.	Mean
Rhizopus oryzae	30	36	0	48	20	0	60	18	2	66	68	80	71.3
R. nodosus	48	12	2	32	22	0	66	14	0	62	54	80	65.3
R. nodosus (b)	32	18	0	28	14	2	58	14	0	50	44	72	55.3
R. nigricans	24	16	4	38	4	0	64	10	0	44	42	74	53.3
R. tritici	22	22	0	22	14	2	62	2	0	44	38	64	48.7
R. chinensis	36	24	2	12	16	4	36	10	0	62	32	46	46.7
R. maydis	34	16	2	24	16	0	42	6	0	52	40	48	46.7
R. arrhizus	32	18	0	32	12	2	34	8	0	50	46	42	46.0
R. microsporus (+)	8	12	4	12	22	2	26	18	0	24	36	44	34.7
R. pyriformis	22	14	2	14	10	0	24	10	0	38	24	34	32.0
R. artocarpi	16	28	0	16	4	0	24	0	0	44	20	24	29.3
R. microsporus (−)	8	16	4	12	6	0	28	8	2	28	18	38	28.0
R. reflexus (+)	22	18	2	8	6	4	16	8	0	42	18	24	28.0
R. delemar	14	12	0	8	20	0	10	10	0	26	28	20	24.7
R. reflexus (−)	12	4	2	12	6	0	10	4	0	18	18	14	16.7
Aspergillus niger	16	12	28	24	28	26	32	16	8	56	78	56	63.3
A. flavus	8	10	16	12	10	8	14	2	0	34	30	16	26.7
Penicillium sp.	10	16	2	6	22	6	8	12	0	28	34	20	27.3

[a] 1 = type shown in Fig. 2, B, C, and D.
2 = type shown in Fig. 2, E.
3 = type shown in Fig. 2, F.

little trouble was experienced with contaminations. When a contamination was noted, the dish was discarded and the test repeated.

In this experiment (table 1), *Rhizopus oryzae*, *R. nodosus*, and *R. nigricans* were the three organisms most active in producing scutellum rot. *R. tritici*, *R. chinensis*, *R. maydis*, and *R. arrhizus* were about alike in pathogenicity, and come next in order. Plus and minus strains of *R. microsporus* and *R. reflexus*, respectively, were very much alike in their behavior. Both species were comparatively low in ability to produce scutellum rot.

All the organisms were effective at the three temperatures used. None of the temperatures was high enough to inhibit growth of any of the fungi. The first five organisms in table 1 were most active at 30° C., whereas some others, such as *R. chinensis*, *R. maydis*, and *R. artocarpi*, were most active at 16° C. *R. artocarpi* is the only species in this group that closely resembles *R. nigricans*. It has the same type of growth, the same limitations with respect to high temperature, and has large spores. The spores are apparently a trifle larger and more irregular than in *R. nigricans*. Each species produced 44 per cent infection at 16° C., but at 22 and 30° C. the latter was much more active than *R. artocarpi*.

It has been shown by Harter and Weimer (5) that physiological variations exist between different isolations of *R. nigricans*. The same is probably true of the other species of *Rhizopus*. The two cultures of *R. nodosus*, according to table 1, did not behave exactly alike. There is a probability, however, that this difference may be due to experimental error.

It should be noted that the rot of type 1 predominates in Rhizopus infection, while type 2 is a close second. Type 3 is comparatively rare. Aspergillus infection, on the other hand, readily causes all three types of rots, type 3 predominating at the low temperature used.

Harter, Weimer, and Lauritzen (4) used all these Rhizopus species with the exception of *R. pyriformis* in inoculation trials on sweet potatoes. They found all of them pathogenic with the exception of *R. chinensis* and *R. microsporus*. Lauritzen and Harter (11) came to the conclusion, however, that *R. nigricans* and *R. tritici* are the species primarily responsible for the decay of sweet potato known as soft rot, *R. nigricans* at temperatures between 6° and 20° C. and *R. tritici* at 30° C. and above, the two overlapping between 20° and 30° C.

Isolations from Rhizopus-infected Corn Kernels Tested on Germinator

It has been frequently stated that *Rhizopus nigricans* is the species which occurs most abundantly. References to fruit and vegetable rots due to attacks by this species are numerous. Perhaps the identifications have usually been correct. Harter *et al* (4), however, call attention to " . . . the common error of classifying all such molds as *Rhizopus nigricans* . . ."

Very likely many mistakes of this kind have been made. *R. nigricans* can be rather easily distinguished from other species because of its inability to grow at certain temperatures, and its large spore size. Only *R. artocarpi* falls in the same class and is hard to distinguish from *R. nigricans*. The chief difference observed by the writer in the cultures available, aside from slight spore differences, is that the vegetative portion of *R. artocarpi* grew less actively than that of *R. nigricans*.

In the central part of the corn belt, *Rhizopus* species are the most common cause of scutellum rot on the germinator. In order to determine which particular species occur most commonly, infected kernels showing the Rhizopus mold on the exterior, as in figure 1, were taken from the germinator at different times and from samples of corn obtained from widely separated places. *Aspergillus* spp. and other fungi sometimes cause scutellum rot on the germinator, but in this case only Rhizopus-infected kernels were selected. The plumule and roots were clipped to within about one-half inch from the kernel. Then the kernels were surface-sterilized for five minutes in mercuric chloride according to the method already described. After

TABLE 2.—*Fungi isolated from corn seedlings affected with scutellum rot*

Organisms isolated	No. of isolations from corn germinated on limestone-sawdust germinator for 7 days at 27° C. 132 seedlings	No. of isolations from corn germinated in virgin prairie soil for 30 days at 16° C. 61 seedlings
Rhizopus tritici type	63	.....
Rhizopus nodosus type	60	6
Rhizopus microsporus type	2	.....
Rhizopus spp. (undetermined)	7	.....
Mucor spp.	.....	24
Penicillium spp.	.....	14
Gibberella saubinetii	.....	3
Fusarium moniliforme	.....	2
Fusarium spp.	.....	12
Aspergillus niger	.....	1
Genus undetermined	.....	5

washing in sterile water, the kernels were opened with sterile instruments and a small piece of the infected tissue was placed on potato dextrose agar. From the resulting fungous growth, single spore cultures were made by the dilution plate method, using Beyerinck's agar. A total of 132 isolations were made, each from a separate kernel. The organisms were identified according to the descriptions given by Hanzawa (2, 3) and Lendener (13) and by comparison with the species in culture, the sources of which have already been given. The results are given in table 2.

The taxonomy of all the Rhizopus species is not as clear as one might wish, yet certain species or groups are well defined. All the cultures obtained in these isolations grew luxuriantly at 37° C. This excludes the possibility of any of them being *R. nigricans, R. artocarpi,* or *R. reflexus.* According to Weimer and Harter, their culture of *R. microsporus* did not grow at 37° C., but Drechsler's culture of the same species did grow at that temperature, and according to Lendener it should do so. All the cultures sporulated abundantly at room temperature. This eliminated the possibility of any of them being *R. chinensis, R. arrhizus,* or *R. maydis.*

Two small-spored forms isolated were identified as *R. microsporus* although the sporangial mass was a dark brown color; whereas the culture from Drechsler was more nearly dark gray on the same culture medium. Spores of all the other isolations were of medium size, and most of them could be assigned to one of two definite groups, one of which was classed as *R. tritici,* the other as *R. nodosus.* The former showed no zoning on potato dextrose agar slants, while the latter showed a distinct zone between the mycelial and sporangial masses, as shown in figure 4. When grown in flasks, it was also noted that the former did not grow as tall as the latter. In these characters as well as in all other aspects that were observed, the forms isolated were identical with the cultures of these species which were received from Dr. L. L. Harter. *R. delemar* and *R. oryzae* obtained from the same source are very similar to *R. tritici* and *R. nodosus* in many respects, but their mycelial mats in cultures have a finer texture. Typical nodes, however, were very rare in these cultures of *R. nodosus* on potato dextrose agar. In the many mounts of this fungus observed, the writer saw only two nodes resembling those figured by Lendener (13).

These data seem to indicate that *R. tritici* and *R. nodosus* are the most common organisms causing scutellum rot on the germinator. In the pure culture inoculations it was shown that *R. nigricans* is a very active causal agent of scutellum rot, but in 132 isolations of *Rhizopus* on the germinator it was not found. The writer does not wish to infer that it is never found in this connection, but it at least seems evident that in the central part of the corn belt it is not usually the predominating agent.

Isolations from Scutellum Rot-susceptible Kernels Grown in Soil

A number of ears of dent corn representing several varieties and sources were selected that averaged about 70 per cent susceptibility to scutellum rot on the germinator. A composite lot of 100 kernels from these ears was planted in virgin soil in a greenhouse where the temperature was maintained very close to 16° C. After 30 days the seedlings were taken from the ground and all soil carefully washed off. Isolations were then made in the manner previously described.

Of the 100 kernels planted, 61 showed scutellum rot infection. Although Rhizopus species are the principal cause of scutellum rot on the germinator, in this case only 6 kernels, or about 10 per cent (see table 2), were infected with *Rhizopus*. On the other hand, there were higher percentages of infection with *Mucor*, *Penicillium*, and *Fusarium*. Cultures of *Gibberella saubinetii* and *Aspergillus niger* were also isolated. All types of infection shown in figure 2 were found. In most cases the rot seems to have started in the epithelial region, just as it usually does on the germinator.

FACTORS ASSOCIATED WITH DISEASE RESISTANCE OR SUSCEPTIBILITY

Stage of Maturity

During the season of 1924, yellow dent corn of the utility type (10) was gathered in the milk, glaze, dent, mature, and husking stages by Mr. S. S. Carney.[3] Germination tests of this corn for vigor and freedom from disease were made by the writer. The ears were dried in a well ventilated warm room. There were 70 ears in each group, and 10 kernels were tested from each ear. In the following season similar groups, with the exception of ears

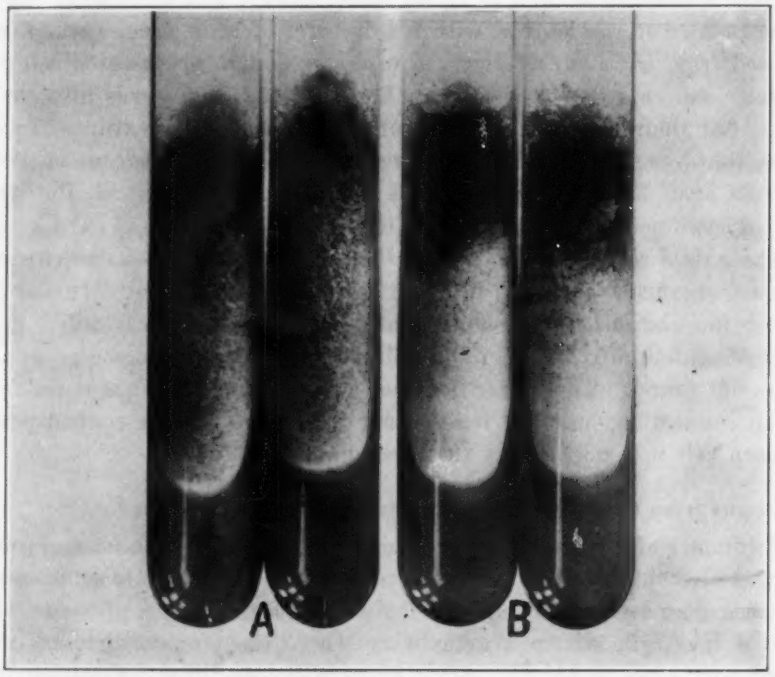

FIG. 4. A comparison of *Rhizopus tritici* (A) and *R. nodosus* (B) in culture on potato dextrose agar slants.

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in the milk stage, were gathered, cured and tested for disease. The percentages of scutellum rot are given in table 3. The ears that were harvested in the milk stage in 1924 had only 6.9 per cent dead kernels, and the kernels from the same ears produced 42.5 per cent strong seedlings. With advancing maturity there were fewer dead kernels and more strong seedlings. In 1925 the highest percentage of dead kernels was 2.3, in the glaze stage. In both seasons the vigor of germination increased with maturity up to the dent stage, where it attained its maximum.

The percentage of scutellum rot was very high in the corn harvested in the milk and glaze stages. It was lower in the dent stage, and very much lower in the mature stage. While the maximum vigor had been attained in the dent stage, susceptibility to scutellum rot was still very high as compared with the more mature stage. Some important changes relative to resistance to the scutellum rot disease occurred during that time.

Similar data were obtained from another lot of yellow dent corn which was harvested at three different stages of maturity. There were 72 ears in each lot. The percentages of scutellum rot were as follows: glaze stage, 56.9 per cent; dent stage, 45.8 per cent; and mature stage, 32.4 per cent. Mature corn therefore is more resistant to this disease than immature corn.

Character of the Endosperm

In speaking of the character of the endosperm in this paper, only the outward appearances with respect to starchiness were considered. The separations were made into classes as illustrated by Trost (16). This same author has found starchy seed corn to be inferior to horny seed in its field performance. Starchy seed also showed the highest percentage of infection on the germinator, the causal organisms mentioned being *Fusarium* spp., *Diplodia zeae*, and *Penicillium* spp.

Holbert *et al* (8) have shown that starchy seed is more likely to be diseased than horny seed when tested on the germinator, and that, when nearly disease-free starchy seed was compared with nearly disease-free horny seed in field tests, greater reductions in yield occurred from the former seed when it was inoculated at planting time with *Gibberella saubinetii* or when it was planted on disease-infested soil.

It was shown by Koehler and Pettinger (10) that, in groups of corn differing in respect to endosperm composition, scutellum rot was most prevalent in the starchy groups. Other corn diseases found in seed corn on the germinator were not influenced by kernel composition in a similar manner.

The ears gathered at different stages of maturity, data on which are given in table 3, were classified with regard to the composition of the endosperm. The corn gathered in the milk stage was very starchy, most of it being of the A class according to Trost's classification. Of the ears har-

vested in the glaze stage, most were in the C class. The amount of scutellum rot infection was practically the same in the first two groups. In the dent stage most of the ears were in class E, and the amount of scutellum rot diminished about 20 per cent. In the mature and husking stages the ears were a little more horny and the percentage of scutellum rot was considerably lower. In general, disease resistance increased with an increase in horniness of the endosperm, but the two were not exactly parallel.

TABLE 3.—*The relation of kernel composition and percentage of scutellum rot in corn harvested at different stages of maturity and classified into six classes, "A" denoting very starchy endosperm, and "F" very horny endosperm*

Year	Stage of development	Number of ears in each class						Scutellum rot in per cent
		A	B	C	D	E	F	
1924	Milk	45	17	8				53.2
	Glaze	5	16	24	15	7	3	54.2
	Dent		2	6	9	34	14	43.5
	Mature			4	14	36	16	20.5
	Husking				14	40	16	19.4
1925	Glaze	4	20	25	31	9	1	21.3
	Dent		1	8	14	36	22	14.1
	Mature				12	39	28	8.4
	Husking				1	25	18	6.1

Data on another group of yellow dent corn ears show a close correlation between endosperm composition and scutellum rot (table 5). All of these ears were mature when harvested. There were variations in endosperm composition and, after the germination tests were made, the ears were divided into four classes with respect to starchiness, namely: C, D, E, and F. There was none that classified as A or B. Percentages of scutellum rot in the different classes were as follows: C, 47.4; D, 39.2; E, 34.0; and F, 29.1. The correlation between the two factors here is very close.

From data obtained and observations made during a number of years, it is evident that starchy seed is much more susceptible to scutellum rot than horny seed. Furthermore, as far as resistance or susceptibility is evident on the germinator, no other disease is similarly influenced by variations in starchiness of the kernels. In the soil, starchy seed also is more susceptible to disease, but whether here it is entirely a scutellum rot relationship is not known. Several soil fungi, however, were isolated from typical scutellum rot conditions in seedlings grown in soil (table 2).

Curing Conditions

The principal object of these experiments was to determine whether a greater degree of disease resistance could be obtained by drying the corn more rapidly than is ordinarily done. No experiments were conducted under poor curing conditions.

In one experiment a lot of ears were divided into four similar groups of 54 ears each. One lot was dried at room temperature ranging from 20° to 25° C. The other lots were dried in hot air currents maintained at temperatures of 39.5° to 45.0°, 42.0° to 47.5°, and 47.5° to 56.5° C. The drying process at these temperatures was extended over a period of 48 hours.

When tested for susceptibility to scutellum rot, the various groups were nearly the same. No benefit was derived through rapid drying.

TABLE 4.—*Effect of different drying conditions on the subsequent susceptibility of yellow dent corn to the scutellum rot disease*

Drying conditions	Percentage Moisture				Percentage of scutellum rot	
	Oct. 21	Oct. 28	Nov. 4	Jan. 24	Without inoculation	After inoculation with *R. nodosus*
In well ventilated room at about 22° C. (240 ears)	34.2	14.9	12.1	11.9	12.8	34.6
Under roof at outdoor temperature (240 ears)	34.0	25.1	18.5	14.8	8.3	34.3

In another experiment 480 ears were harvested in the mature stage on October 21, 1924, from green, apparently healthy stalks. Half of these were laid in wire hangers and hung in one of the farm buildings. The windows were kept open so as to allow plenty of ventilation. The other half of the ears was placed into similar hangers, but hung in a heated building. Some of the windows were also kept partly open during the first month to permit ventilation. Moisture tests were made of this corn at the time of harvest and at three later periods. The data are shown in table 4. The initial moisture content was 34.0 to 34.2 per cent. In the warm room this was reduced to 14.9 per cent during the first week. At the outdoor temperature three months were required to dry the corn to the same extent. When disease readings were made on the germinator in the ordinary way, the corn that was dried quickly showed the most scutellum rot. But when all the kernels were sprayed with a spore suspension of *Rhizopus,* the disease readings were practically identical. Evidently the corn dried indoors was more subject to contamination with Rhizopus spores; but when all of it was inoculated so that all had equal opportunities, it became evident that

these two methods of curing had no differential effect on the corn's resistance to scutellum rot.

Diffusions from Germinating Corn Kernels

Observations on the germinator (Fig. 1) and under pure culture conditions have shown that the molds grow much better on some corn kernels than on others. As *Rhizopus* belongs to a group of organisms known as weak parasites, it seemed likely that the organisms did not need to penetrate into the scutellar region before coming into contact with their food supply, but that during the germinating process food materials were diffused from these kernels which gave the fungi their initial start. Consequently some experiments were planned to determine whether (*1*) during the germinating process substances were diffused from the kernels which support growth of organisms that may cause scutellum rot; (*2*) whether there is any difference in this respect between seedlings resistant and susceptible to scutellum rot; and (*3*) if there is a difference, to determine whether it is due to the relative amount of total matter diffused or whether it is due rather to the specific character of these substances.

Petri dishes were prepared with sterile non-nutrient agar. Test dishes inoculated with *R. nodosus* developed no growth beyond the spore germination stage. Disease-free, surface-sterilized kernels of corn susceptible to scutellum rot were placed in one set of dishes, five kernels to a dish. Disease-free, resistant corn kernels were treated similarly and placed in another set of dishes. This corn was germinated at room temperature until the plumules were about two inches long. Then all the seedlings were removed from the dishes, and spores of *R. nodosus* were sown on the agar.

Considerable fungous growth occurred in the plates in which the susceptible seed had germinated (see Fig. 5), but very sparse growth occurred in those plates in which the resistant kernels had germinated. This showed that the substances passing out from the kernels during the germinating process supported Rhizopus growth well in one case, but very poorly in the other. This indicated two possibilities: either considerable food material passed out in the one case and very little in the other, or food materials passed out in both cases, but a toxic substance was also present in the agar upon which the resistant seed was germinated.

Corn kernels were germinated in flasks of distilled water. It was found that when resistant seed was used, the water became slightly turbid; but when susceptible seed was used, the water became considerably more turbid. When the kernels were partly submerged, they germinated very well; when entirely submerged, they did not germinate. It was found that the turbidity was greater when the kernels were allowed to germinate than when they did not germinate. Neither the kernels nor the water was sterilized. By

evaporating the solutions to dryness, it was also found that more solid matter had diffused from the germinated kernels than from those that had not germinated.

A group of seed ears was divided into four classes with respect to starchiness. (See table 5.) Class C was somewhat starchy, while Class F was very horny, as already explained. In each group, the 10 most susceptible and the 10 most resistant ears were selected. Representative kernels from these 10-ear groups, as well as from the groups as a whole, were taken for making extractions by the diffusion method. Twenty grams of corn kernels and 70 cc. distilled water were placed in 150 x 20 mm. petri dishes. They were made up in triplicate and were incubated for three days at 30° C. Good germination of the corn was secured. At the end of that time the liquids were filtered through coarse filter paper (Whatman No. 4) and tested for crystalloids with an Abbe refractometer, and for turbidity with a Kleinman nephelometer. A 0.2 per cent cornstarch solution was used as a standard colloidal solution against which the turbidity of the liquids was

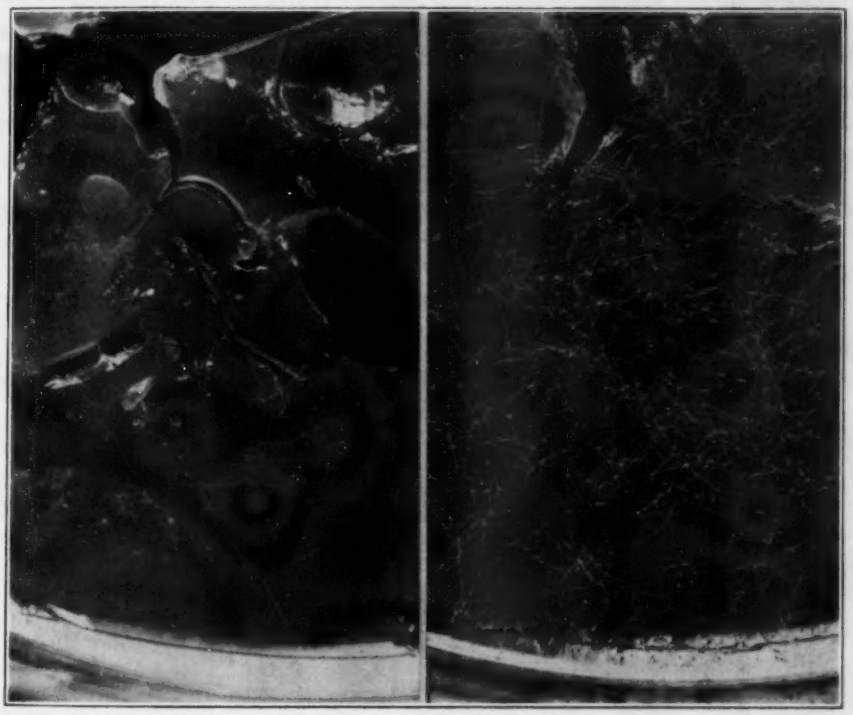

FIG. 5. Rhizopus growth on non-nutrient agar on which, at the left, resistant corn kernels had germinated and, at the right, susceptible kernels had germinated. The kernels were removed before inoculation with *Rhizopus*. The fungus grew on the nourishment diffused from the kernels. Enlarged.

checked. The solution was made by boiling the starch in distilled water for six hours under a reflux condenser, adding mercuric chloride as a preservative, allowing it to stand for a week, and decanting the upper liquid. The results of these readings as well as the percentage of scutellum rot in each group of corn as found on the germinator are given in table 5.

The refractometer showed no differences between the solutions from the various corn selections. There were only small amounts of solutes in the solution. A check reading made with pure water registered an index of 1.338 under the same temperature conditions.

The nephelometer readings proved very interesting. It must be borne in mind that the lower the scale reading the higher the turbidity of the solution. The lowest reading (highest turbidity of the solution in which the corn was germinated) was practically equivalent to a 1.0 per cent starch solution. It should be noted first that within each grade of starchiness the most turbid solutions came from the susceptible seed and the least turbid from the resistant seed. Furthermore, with decreasing soft starch in the endosperm, both disease susceptibility and turbidity of the liquid decreased.

Microscopic examination showed that the turbidity was due, at least in large measure, to bacterial flora. The experiment was repeated except that the corn kernels were first sterilized according to the method already described, and the diffusions were made under sterile conditions. No turbidity occurred in the solutions. Ears infected with *Fusarium moniliforme*, how-

TABLE 5.—*Refractometer and nephelometer readings of culture solutions and percentages of scutellum rot in four lots of corn, differing with respect to degree of starchiness*

Grade of starchiness	Ear selections	Number of ears	Refractometer index	Nephelometer readings in mm.		Scutellum rot in per cent
				I	II	
C	Most susceptible	10	1.3342	5.5	5.0	79.0
	Whole sample	47	1.3342	6.0	5.2	47.4
	Most resistant	10	1.3341	8.5	8.6	27.0
D	Most susceptible	10	1.3341	7.3	6.0	82.0
	Whole sample	97	1.3342	8.5	8.0	39.2
	Most resistant	10	1.3340	17.0	15.0	5.0
E	Most susceptible	10	1.3343	6.0	7.0	76.0
	Whole sample	257	1.3342	10.0	10.5	34.0
	Most resistant	10	1.3341	11.5	11.7	3.0
F	Most susceptible	10	1.3342	8.5	9.5	68.0
	Whole sample	85	1.3341	13.5	10.7	29.1
	Most resistant	10	1.3341	15.7	13.5	2.0

ever, had to be rejected because a 90-minute treatment with a 0.5 per cent Uspulun solution does not kill this fungus. These results seemed to demonstrate that the turbidity was due entirely to living organisms and not to materials diffused from the kernels.

Again, it became evident, just as in the previous experiment where kernels were germinated on non-nutrient agar, that in the case of the scutellum rot-susceptible kernels, something diffused which was favorable to the growth of organisms, in this case bacteria; whereas from resistant seed, either very little diffusion of materials suitable for bacterial growth occurred, or some chemical inhibitor was present.

An experiment was planned in which the total amount of solid non-volatile material diffused from the susceptible and resistant kernels could be compared. Eight ears each of five kinds of disease-free corn were selected, namely (1) Reid's Yellow Dent resistant to scutellum rot, horny endosperm; (2) Reid's Yellow Dent susceptible to scutellum rot, horny endosperm; (3) Reid's Yellow Dent, susceptible, but with starchy endosperm; (4) an inbred line, resistant, and (5) an inbred line susceptible to this disease. Some information on the history and field performance of these inbred strains has already been published (9, pp. 354–6). The two inbred strains compared here are very much alike in size, shape, and texture of kernels.

As in the previous work, 20 grams of corn were placed in 70 cc. distilled water in large petri dishes and allowed to germinate under sterile conditions. Three dishes were made up from each ear. After 3 days in the case

TABLE 6.—*Weights of extracted solid matter from five kinds of corn, the extractions having occurred while 60 grams of corn were germinated in 210 cc. distilled water under sterile conditions*

Ear No.	Dry weight in grams of extracted solid matter				
	Resistant Reid's Yellow Dent, horny endosperm	Susceptible Reid's Yellow Dent, horny endosperm	Susceptible Reid's Yellow Dent, starchy endosperm	Resistant Pure line No. A–1–1–2–R–1–1–9–1	Susceptible Pure line No. A–1–1–4–1–8–1–5–1
1	0.192	0.150	0.453	0.791	0.163
2	0.257	0.177	0.648	0.822	0.190
3	0.322	0.203	0.364	0.817	0.231
4	0.307	0.195	0.429	0.762	0.196
5	0.230	0.245	0.520	0.812	0.210
6	0.214	0.235	0.378	0.848	0.244
7	0.196	0.210	0.407	0.743	0.132
8	0.202	0.185	0.485	0.794	0.171
Averages	0.240	0.200	0.461	0.799	0.192

of the Reid's Yellow Dent corn and 4 days in the case of the inbred corn, the solutions from all three dishes were transferred to a beaker and evaporated to a small volume at 85° C. This solution was then transferred to a tared weighing bottle and dried down to constant weight. The results are given in table 6. Not all of these could be handled at one time. Only two ears of each of the five kinds of corn were used at each operation.

It is evident that the total matter diffused from susceptible seed is not greater than that from resistant seed when both groups are similar in respect to horniness of the endosperm. The starchy seed did show a uniformly greater amount of solid material in the culture solution than did the horny seed. This may account in part for the fact that starchy seed usually is more susceptible than horny seed.

In the inbred strains the amount of solid matter in the culture solution from the susceptible line was even very much less than from the resistant line. This is the reverse of what would occur if resistance or susceptibility

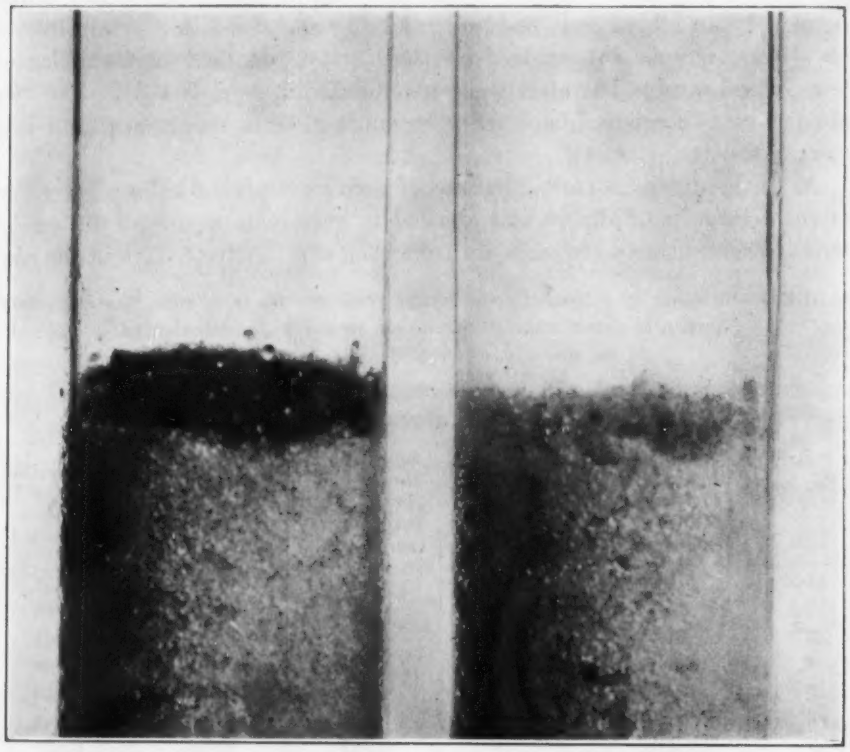

FIG. 6. Rhizopus growth on corn extracts; left, from susceptible seed; right, resistant seed. The extracts were made by germinating the corn in sterile water. These solutions were added to sterile sand in test tubes and then inoculated with *Rhizopus*. Enlarged. Note the difference in growth.

were governed by the amount of material that diffuses from the germinating kernels. Therefore it is probably not the quantity of this material but its specific character that influences the expression of resistance or susceptibility to the disease.

In another experiment, sterile culture solutions from the resistant and susceptible inbred lines were placed on heat-sterilized sand in test tubes, 5 cc. liquid to 18 cc. dry sand. These tubes were then inoculated with *R. nodosus*. In the tubes with solutions from susceptible seed the fungus grew well; but in the other tubes, although the amount of solid matter diffused out was greater, there was very poor growth (Fig. 6). In tubes similarly prepared, but heated in the autoclave for 30 minutes at 15 pounds pressure before inoculating, there was good fungous growth in both sets, the fungus, however, still growing better in the tubes with solutions from susceptible seed. This experiment was repeated, except that resistant and susceptible open-pollinated seed was used. The results were the same.

Something happened during the autoclaving process that changed the extract from the resistant seed. Either something was removed by distillation or some physico-chemical change occurred that made the extract more suitable as a nutrient material for the fungus.

SUMMARY

Scutellum rot of corn is a disease that occurs during the seedling stage of the plant and may be caused by a number of different organisms. On the germinator, *Rhizopus* spp. are the most common causal agents. The initial attack is most common in the epithelial region of the scutellum, but often also occurs at other points.

Inoculations with 12 species of *Rhizopus* at temperatures of 16°, 22°, and 30° C. showed that all were able to cause scutellum rot at these temperatures, but differences in virulence were noted.

Isolations from Rhizopus-infected kernels on the germinator showed that *R. tritici* and *R. nodosus* were the predominating species under the conditions studied. Infection with *R. nigricans* was not found.

Isolations from seedlings grown from susceptible seed in soil at 16° C. showed that species of *Mucor*, *Penicillium*, and *Fusarium* were the predominating organisms associated with scutellum rot. Only about 10 per cent of the diseased kernels were infected with *Rhizopus*.

Immature seed was found to be more susceptible to scutellum rot than mature seed. The former seed was also found to be much more starchy in endosperm texture than the latter. This was perhaps the principal factor concerned.

In mature, field selected, seed corn, the starchy ears were more susceptible to scutellum rot than those with horny endosperm.

Slow drying of corn at outdoor temperature versus rapid drying at various warmer temperatures did not influence the resistance or susceptibility to scutellum rot.

During the germination process, especially after the pericarp breaks, substances diffuse from the kernels into the surrounding water. This occurred with all kernels tested, resistant or susceptible. In the case of susceptible kernels the extract obtained by the diffusion process was a suitable medium for vigorous growth of *Rhizopus*. The extract from the resistant seed induced a very poor growth of *Rhizopus*.

The substances emanating from germinating susceptible kernels, therefore, seem to stimulate vigorous growth of certain organisms on the exterior of the kernel or in the surrounding soil. These would grow most actively in the direction of the greatest concentration of the food substances diffusing from the kernel at the place the pericarp has ruptured. Entry of the kernel is then effected, usually at the margins of the scutellum.

As no substances suitable for fungous growth diffuse from germinating resistant kernels, there is little fungous growth on the kernel and subsequently into the kernel.

When the substances extracted by the diffusion process from susceptible and resistant kernels were evaporated down to dryness, no quantitative difference was found that would account for differences in resistance or susceptibility. The differences in these extractions, therefore, must be qualitative rather than quantitative.

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EFFECT OF EARLY SPRAY AND DUST APPLICATIONS ON LATER INCIDENCE OF CUCUMBER WILT AND MOSAIC DISEASES

E. E. CLAYTON

INTRODUCTION

The bacterial wilt (*Bacillus tracheiphilus* E. F. S.) and mosaic of cucumber are two diseases of major importance that are said to be largely, if not entirely, disseminated by insects. Of the several carriers concerned, Doolittle and Walker (2) found the striped cucumber beetle (*Diabrotica vittata* Fab.) to be the most important in transmitting the virus of mosaic from wild host plants to the cultivated cucumber. Rand and Enlows (3) and Doolittle (1) report that this same insect is responsible for the overwintering and the primary spread of the wilt disease Thus we have a single primary carrier for both diseases. It appears reasonable to suppose that elimination of the chief means by which these diseases are introduced into the cucumber fields would be an effective method of control. How effective, however, seems to be a moot question, since the method has apparently not been tried out to any extent under controlled field conditions.

The present investigation was begun with the object of determining:- (a) the value of the best entomological recommendations for striped beetle control, when faithfully followed, in restricting the occurrence of wilt and mosaic; (b) the effect of the addition of a fungicide (bordeaux) to the insecticide as a means of increasing its disease control value.

EXPERIMENTAL PROCEDURE

The work was carried out during 1925 and 1926 in eastern Long Island, where both diseases are very prevalent and destructive. All spray applications were made by the writer, and the work has been thorough and timely. The first treatments were given immediately after the first beetles appeared and dust treatments were renewed after each rain. The spray treatments were renewed as the growth of the cucumber vines required.

In the 1925 experiment, 27 plats were laid out, each consisting of a single row 66 feet long, with the plants spaced a foot apart. Seed was sown on June 3. The special beetle control treatments were applied during the first 5 weeks of growth. After that time, the plants having reached the vining stage, regular applications of bordeaux spray were given at 5- to 10-day intervals. In 1925 during the initial 5-weeks' period the following treatments were compared.

Series A: Sprayed with bordeaux mixture (2–4–50) + 3 lbs. arsenate of lead and ½ gallon miscible oil for spreader. Five applications were made in all: June 15, 19, 25, 30, and July 6.

Series B: Dusted with arsenate of lead and lime (1 to 15). Seven applications were made: June 16, 19, 22, 26, 29, 30 and July 6.

Series C: Given only sufficient protection to prevent loss of plants from the excessive feeding of the insects. One application of dust was necessary, and was given on June 22.

Treatment A was decided upon as the result of preliminary trials. Treatment B was recommended to the writer by the entomologist as the most satisfactory control for beetles known at the time.

In the 1926 experiment, certain modifications were introduced. First the size of the plats was greatly increased. In place of a single 66-foot row, six rows each of 36-foot length were used. This made a rectangular block 24 feet wide, with each block separated on sides and end by a 12-foot interval. The seed was sown in hills with 12 hills to a row and 3 plants to a hill. The date of sowing was June 17, and the special treatments covered the subsequent 6-weeks period. All plats after this were given regular and uniform applications of bordeaux mixture.

Series A received bordeaux mixture (2.5–5–50) + 3 lbs. arsenate of lead and ½ gallon miscible oil for two applications and then 4–6–50–3–0.5 for two more. The dates of applications were July 7, 12, 19 and 23.

Series B received calcium arsenate-gypsum dust (1–15) for five applications: July 7, 12, 16, 19, and 23.

Series C received a single dusting, merely to keep the beetles from eating the young plants. This was applied on July 12.

The calcium arsenate-gypsum dust was substituted for the lead arsenate-lime combination on the recommendation of the entomologist. The 2–4–50 bordeaux was increased in strength to determine whether better disease control could not be secured. Thus Rand and Enlows (3) recommend a 4–5–50 bordeaux as being better than weaker solutions.

During the two seasons a number of other diseases and insects were encountered, but in no case were they serious factors. The most bothersome was a thrip infection in 1925 which threatened for a short time to become serious. A thorough treatment of nicotine dust applied in the middle of a warm day reduced this trouble very satisfactorily. In 1926 aphids were troublesome, and nicotine was used in one of the general sprays with fairly satisfactory results. The only disease in addition to wilt and mosaic that assumed any serious proportions was powdery mildew caused by *Erysiphe cichoracearum* D. C. This trouble was encountered late in the summer of 1926 and was only partly controlled by bordeaux spraying.

TABLE 1.—*Effect of early spray and dust treatments on the incidence of bacterial wilt of cucumber in 1925*

Plot no.	Variety	Treatment[a]	No. plants killed by wilt	
			July 6	July 17[b]
		SERIES A		
1	Kirby		0	2
2	do		0	2
3	do	Sprayed with bordeaux	0	0
4	Boston Pickle	mixture (2–4–50) plus 3	1	0
5	do	lbs. lead arsenate plus ½	2	0
6	do	gal. oil. Five applica-	0	0
7	Chicago Pickle	tions.	0	1
8	do		0	0
9	do		0	1
			Total	9
		SERIES B		
1	Kirby		0	0
2	do		0	2
3	do		1	3
4	Boston Pickle	Dusted with arsenate of	2	0
5	do	lead and lime (1–15).	0	2
6	do	Seven applications.	0	3
7	Chicago Pickle		0	1
8	do		1	0
9	do		0	0
			Total	15
		SERIES C		
1	Kirby		1	14
2	do		6	7
3	do		6	9
4	Boston Pickle	Checks. One application	3	6
5	do	of dust to prevent excess	5	4
6	do	feeding of insects.	3	4
7	Chicago Pickle		2	6
8	do		4	9
9	do		1	7
			Total	97

[a] In each plot one 66-foot row of plants, one foot apart, was treated.

[b] The disease continued active in Series C after this date, but further counts were not made.

TABLE 2.—*Effect of early spray and dust treatments on the incidence of cucumber mosaic in 1925*

Plot no.	Variety	Treatment[a]	No. pickles picked									
			July 27		July 30		Aug. 3		Aug. 7		Aug. 11	
			H[b]	M	H	M	H	M	H	M	H	M
		SERIES A										
1	Kirby	Sprayed with bordeaux mixture (2–4–50) plus 3 lbs. arsenate of lead plus ½ gal. oil. Five applications.	35	1	51	0	78	6	55	34	40	27
2	do		29	0	23	2	42	8	44	32	38	25
3	do		31	2	28	3	62	19	55	21	31	28
4	Boston Pickle		22	3	35	2	30	9	25	54	18	32
5	do		15	0	26	0	45	5	32	33	36	38
6	do		11	0	22	0	59	9	41	20	23	27
7	Chicago Pickle		20	1	29	2	32	10	29	36	29	26
8	do		31	2	47	2	59	9	30	36	42	32
9	do		20	1	26	1	44	14	35	28	19	21
Totals			214	10	287	12	451	89	346	294	276	256
Average percentage mosaic			4		4		16		46		48	
		SERIES B										
1	Kirby	Dusted with arsenate of lead and lime (1–15). Seven applications.	8	4	25	0	37	1	29	22	38	34
2	do		17	2	14	1	31	5	28	11	36	22
3	do		21	0	55	2	49	12	50	41	36	26
4	Boston Pickle		16	9	20	7	24	21	12	28	28	45
5	do		16	0	14	0	40	14	15	20	27	31
6	do		14	0	22	5	44	13	19	36	28	22
7	Chicago Pickle		15	7	26	3	30	10	26	29	28	22
8	do		24	1	33	1	35	8	28	23	17	26
9	do		21	0	27	1	34	8	30	28	28	26
Totals			152	23	236	20	324	92	237	248	266	254
Average percentage mosaic			13		8		22		51		49	
		SERIES C										
1	Kirby	Checks. One application of dust to prevent excess feeding of insects.	21	1	14	7	15	10	12	33	10	12
2	do		4	0	10	3	9	6	11	13	21	13
3	do		10	1	4	1	11	2	4	15	10	20
4	Boston Pickle		23	0	6	1	16	14	7	31	6	25
5	do		0	1	2	1	7	3	11	5	6	3
6	do		4	0	6	1	7	7	7	4	15	8
7	Chicago Pickle		14	9	19	2	14	12	7	28	13	24
8	do		5	2	12	0	11	9	20	16	21	14
9	do		13	0	12	0	8	9	15	13	13	9
Totals			94	5	85	16	98	72	94	158	115	128
Average percentage mosaic			5		16		42		62		53	

[a] In each plot one 66-foot row of plants one foot apart was treated.
[b] H denotes healthy pickles; M denotes mosaic pickles.

TABLE 3.—*Effect of early spray and dust treatments on the incidence of bacterial wilt of cucumber in 1926*

Plot no.	Variety	Treatment[a]	No. plants killed by wilt	
			July 29	Aug. 12
		SERIES A		
1	Kirby	Sprayed with bordeaux mixture (2.5–5–50) plus 3 lbs. lead arsenate plus ½ gal. oil. Two applications. Bordeaux mixture (4–6–50) plus same amounts of lead arsenate and oil. Two applications.	0	3
2	do		0	0
3	Boston Pickle		0	0
4	do		0	1
		Total		4
		SERIES B		
1	Kirby	Dusted with calcium arsenate and gypsum (1–15). Five applications.	0	1
2	do		0	1
3	Boston Pickle		0	4
4	do		0	4
		Total		10
		SERIES C		
1	Kirby	Check. One application of dust to prevent excess feeding of insects.	2	21
2	Boston Pickle		5	24
		Total		52
		Checks doubled for comparison		104

[a] Each plot consisted of six rows 36 feet long. The plants were grown in hills 3 feet apart with 3 plants to the hill.

DISCUSSION OF RESULTS

In considering the four tables it should be pointed out first that wilt was very prevalent in 1925 and 1926 but especially so in 1925. Mosaic was very severe in 1925 but much less so in 1926. Consequently for the latter year totals only are given of the mosaic pickle counts (table 4). Infections were too few to make the data for individual pickings significant, as was possible in 1925.

Wilt Control.—The effective control of bacterial wilt both by spray (Series A) and dust (Series B) was striking each year, although the spray gave slightly better results. Since the difference, however, is not large when contrasted with the amount of disease in the check plats (Series C), it may be said that spray and dust were about equally satisfactory. It

TABLE 4.—*Effect of early spray and dust treatments on the incidence of cucumber mosaic in 1926*

Plot no.	Variety	Treatment[a]	No. healthy pickles picked									Total no. mosaic pickles
			August				September					
			12	16	20	27	3	7	10	13	17	
		SERIES A										
1	Kirby	Sprayed with bordeaux mixture (2.5–5–50) plus 3 lbs. lead arsenate plus ½ gal. oil. Two applications. Bordeaux mixture (4–6–50) plus same amounts of lead arsenate and oil. Two applications.	85	135	101	184	225	63	167	116		4
2	do		115	178	116	340	266	117	168	176		17
3	Boston Pickle		51	140	53	137	139	115	213	187	78	1
4	do		59	151	44	147	135	118	139	173	87	7
	Totals		310	604	314	808	793	413	687	652	165	29
		SERIES B										
1	Kirby	Dusted with calcium arsenate and gypsum (1–15). Five applications.	199	209	173	360	289	97	187	130		32
2	do		164	201	110	336	259	136	150	153		46
3	Boston Pickle		55	118	38	142	94	112	106	150	94	28
4	do		99	144	44	160	120	106	124	159	60	6
	Totals		517	672	365	998	762	451	567	592	154	112
		SERIES C										
1	Kirby	Check. One application of dust to prevent excess feeding of insects.	73	115	55	235	181	97	127	113		61
2	Boston Pickle		48	107	39	122	65	69	98	112	56	17
	Totals		121	222	94	357	246	166	225	225	56	78
Checks doubled for comparison			242	444	199	714	492	332	450	450	112	156

[a] Each plot consisted of six rows 36 feet long. The plants were grown in hills 3 feet apart with 3 plants to the hill.

appears, in sections where wilt is a problem, that it is essential in controlling the striped beetle to consider more than the immediate damage due to its feeding. In these experiments the check plats (Series C) were given sufficient protection to prevent loss of plants through the feeding of beetles, but this degree of protection failed as a wilt preventive.

The early gathering and destruction of diseased plants is usually recommended as an important control measure for wilt. All wilt-infected plants found on the first inspection were removed but at the time of the second inspection the plants were too large and intertwined for this to be done. The removal of diseased plants has not appeared to have any noticeable effect upon the subsequent development of the disease, and the data from Series C indicate that it is of little value as a control measure.

Rand and Enlows (3) summarize their chief recommendations for field control of the wilt disease as follows:

> "Where the disease is likely to be severe, spraying with strong bordeaux mixture and lead arsenate powder (4–5–50–2) is recommended. Treatments should begin as soon as the cucumber plants develop their first true leaves and should continue at intervals of about a week until the cucumber beetles practically disappear from the field. In localities where downy mildew is also prevalent the treatments should also be continued later as a partial insurance against this disease.
>
> "The pulling of wilted vines during the first part of the season, or as long as it can be done without mechanically injuring the healthy plants, will greatly assist in controlling bacterial wilt if consistently done in all neighboring fields. The diseased vines should be buried or otherwise removed from access by the beetles."

In the experiments here reported, the use of a bordeaux mixture stronger than 2–4–50 on young plants caused injury and reduced the yield. The wilt control secured with this weaker mixture was almost perfect, although in one year experimental plats were adjacent to a field alive with beetles and severely infested with wilt. The conclusion is that, if by careful spraying or dusting the striped beetles are effectively controlled, there will be very few wilt-diseased plants needing removal; if, on the other hand, due to lack of attention, the beetles are permitted to feed freely and spread the disease, no subsequent attention to the removal of diseased plants will prevent serious loss from the disease. Either spraying or dusting will satisfactorily control wilt if the work is thoroughly done.

Mosaic Control.—The results of 1925 indicated that the early sprays (Series A) temporarily checked the development of the mosaic disease. Thus for the first three pickings the sprayed plants average 4, 4 and 16 per cent mosaic; the dusted plants 13, 8 and 22 per cent mosaic; the check plants 5, 16 and 42 per cent mosaic. Later, however, the disease became general and these differences disappeared. The results of 1926 paralleled those of 1925. This year less than one-fifth as many mosaic pickles were gathered

from sprayed plants as from dusted or check plants. Taking the matter as a whole, it appears that the early sprays (Series A) were partly effective in preventing mosaic infection and the early dust treatments (Series B) had but little influence. However, the results of 1925 indicate that the degree of control which has been secured in these experiments is not sufficient to give any real protection against the mosaic disease since the disease spreads rapidly and sufficient infection occurs in spite of the early treatments to cause a maximum loss.

Effect of Spray or Dust Treatments on Growth and Yield.—In 1925 the bordeaux-sprayed plats out-yielded the dusted plats. The difference was most prominent in the early pickings. Thus the first picking from the nine dusted plats yielded about one-fourth less than the first picking from nine sprayed plats. This difference in yield was not correlated with any apparent differences in size of vines. The lime-arsenate of lead dust is, of course, liable to cause stunting if applied too heavily, and care was taken to apply it lightly and uniformly. Nevertheless the dusted plants did not have as good color as those which had been sprayed, they did not yield so well, and they began to die sooner in the autumn.

In 1926 a stronger bordeaux mixture was used and gypsum-calcium arsenate was used as a dust. The stronger bordeaux proved toxic and stunted the vines. The stunting was not extremely pronounced but could be easily detected. The dusted plats distinctly out-yielded the sprayed plats this year in the early pickings but even with this handicap the sprayed plats out-yielded the dusted in the last two pickings. The color of foliage in the dusted plats was as good as the color of the plants on the sprayed plats during the first two-thirds of the season, but this year again the dusted vines began to die first. This tendency of the dusted vines to die first in the fall is interesting in view of the fact that after the early treatments for beetle control all plats were given uniform bordeaux spray treatment for the remainder of the season. In 1926 it appeared that the early death of plants on the dusted plat was associated with greater susceptibility to powdery mildew.

SUMMARY

The chief primary agency for dissemination of the bacterial wilt and mosaic disease of cucurbits is the striped beetle, and the control of this insect requires applications of dust or spray during the first six weeks after planting.

Bacterial wilt may be effectively controlled by spraying with bordeaux mixture and lead arsenate or by dusting with lead arsenate-lime dust or calcium arsenate-gypsum dust. A thorough program of spraying or dusting to control beetles is a practical method of preventing loss from bacterial wilt.

The bordeaux and lead arsenate spray reduced the amount of mosaic distinctly; the dust treatments reduced the mosaic slightly; but none controlled mosaic satisfactorily.

The 2–4–50 bordeaux spray proved distinctly better than a stronger mixture, since the latter caused stunting and reduced yield. The calcium arsenate-gypsum dust (1–15) was a very satisfactory mixture.

There was a distinct tendency of plants given early application of bordeaux to remain green longer in the fall than plants given the dust treatments.

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THE EFFECT OF DIFFERENT HOSTS UPON THE SPORANGIA OF SOME PHYTOPHTHORAS[1]

LEON H. LEONIAN

The genus *Phytophthora* is composed of some highly plastic fungi. Many morphological as well as physiological characteristics of these organisms are governed to a great extent by the environmental conditions. This fact has been sufficiently demonstrated by laboratory experiments. Environment, either in the laboratory or in nature, is nothing more than a combination of chemical and physical factors. Since a host plant also exerts such chemical and physical influences, it is to be concluded that, if an organism can be modified by laboratory conditions, there is no reason why similar modifications should not follow in nature, where changes are more abrupt and food factors more complex. With this idea in view the writer inoculated a number of fleshy fruits and vegetables with some eighty different cultures of *Phytophthora*. Green bananas, oranges, lemons, potatoes, tomatoes, peppers and egg-plants were successfully inoculated. About 90 per cent of the organisms were able to infect all of these hosts through wounds and to produce a rapid deterioration of the tissues. This omnivorous habit shows how futile it is to use host relationships as limiting factors in the specific differentiation of Phytophthoras. The symptoms in the majority of cases were uniformly alike. Sherbakoff (4) observed a uniform similarity of symptoms when he inoculated various hosts with *Phytophthora omnivora* (*P. terrestris*).

In spite of the ready infection of all hosts, many of the organisms produced sporangia in great abundance only on tomatoes, egg-plants and peppers. Whenever these reproductive bodies occurred, however, there were striking differences in their morphological features, their size and shape, the nature of their papillae, etc.

Fig. 1 shows the sporangia of *P. phaseoli* as found on the pods of lima-bean and on bell-peppers. The pepper was inoculated directly from the bean. These and subsequent drawings were made with camera lucida, and no attempt was made to select any unusual type; the proportions, therefore, are relatively correct.

Fig. 2 represents the sporangia of *P. capsici* as they were produced upon the following substrata: potassium nitrate solution, egg-plant, bell-pepper, and tomato. To secure fruiting under controlled conditions, a well-nour-

[1] Published with the approval of the Director of the West Virginia Experiment Station as Scientific Paper No. 35.

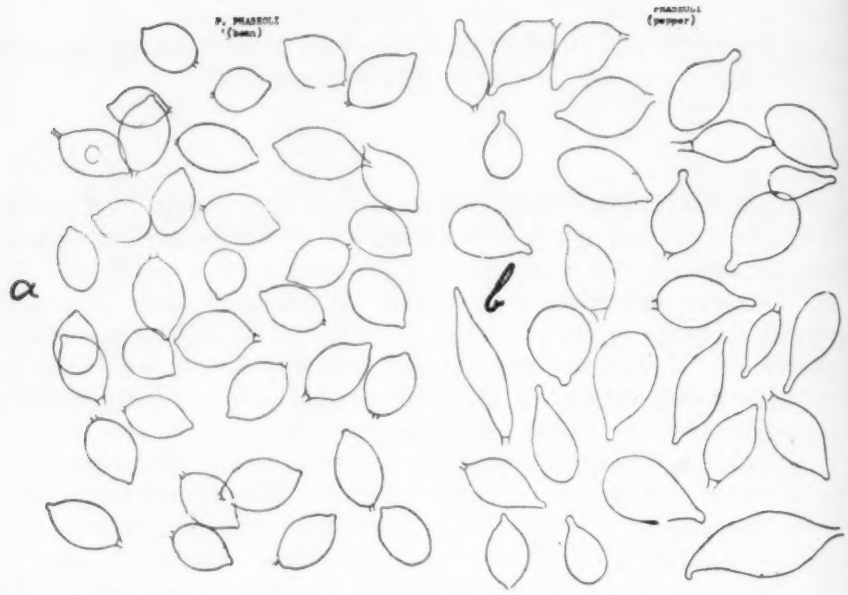

FIG. 1. Sporangia of *Phytophthora phaseoli*. a, on lima bean; b, on bell-pepper.

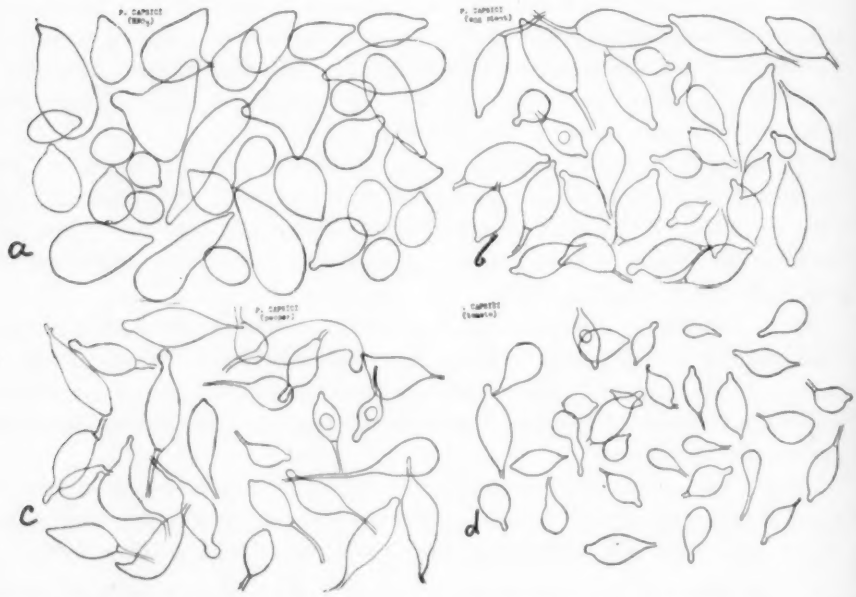

FIG. 2. Sporangia of *Phytophthora capsici*. a, on potassium nitrate solution; b, on egg-plant; c, on bell-pepper; d, on tomato.

ished mycelium was washed in sterile distilled water and transferred to 2 cc. of M/100 potassium nitrate solution. After being kept at 25° C. for three days, the culture was examined for the reproductive bodies and the drawings were made. In case of host plants, sporangia were secured two days after the initial appearance of these bodies. This practice insured the exclusion of immature sporangia to a great extent.

Figs. 3, 4 and 5 represent the sporangia of three strains of *P. omnivora*

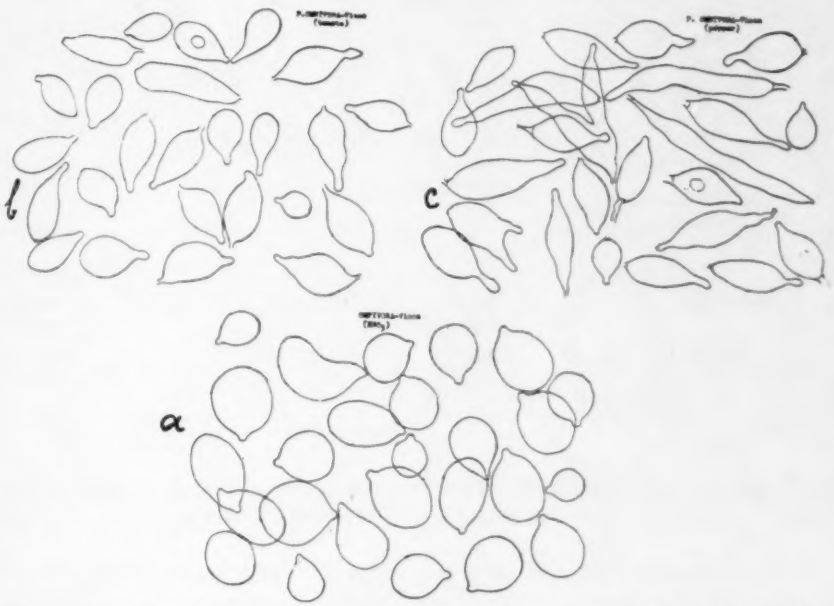

FIG. 3. Sporangia of *Phytophthora omnivora* (Vinca strain). b, on tomato; c, on bell-pepper; a, in potassium nitrate solution.

which were originally isolated from *Vinca*, tobacco and tomato respectively. This species is the most common and the most widespread representative of the genus. It is perhaps for this reason that a number of strains of this fungus were described as separate species by different workers, who felt themselves bound by the time-honored morphological standards. A glance at the various figures is sufficient to substantiate this statement.

Not only the different species of a given host plant but even two varieties of the same species exert entirely different influences upon the sporangia. Fig. 5 shows the tomato strain of *P. omnivora* "performing" on different hosts. On the mild bell-pepper the sporangia are very much elongated, and the papillae in most cases are drawn out thread-like. What a contrast between these and the reproductitve bodies formed on the "hot" pepper! Fig. 6 shows the sporangia of *P. mexicana*. The differences are obvious.

The sporangia are very small and spherical on the tomato, very large and very elongated on the "hot" pepper; and what substantial papillae on the reproductive bodies formed on bell-pepper!

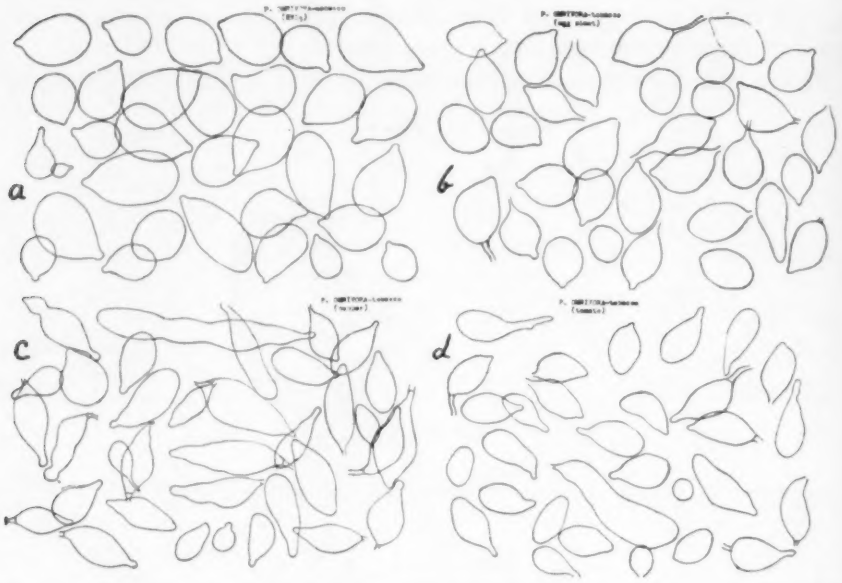

FIG. 4. Sporangia of *Phytophthora omnivora* (tobacco strain). a, in potassium nitrate solution; b, on egg-plant; c, on bell-pepper; d, on tomato.

It was observed that not only the different varieties of the same host but even different strains of the same variety were able to induce morphological modifications. This, however, is believed to be caused by the fluctuations in the environmental conditions rather than by the differences in the chemical composition of the host strains. Too much moisture, for example, checks formation of sporangia; a fairly abundant supply causes the production of larger sporangia; while a restricted supply of air moisture is conducive to much smaller reproductive bodies. The type of different saprophytes following the primary infection, and the nature of their by-products are probably important limiting factors in the morphological changes of the sporangia.

It may be deduced from the foregoing data that, so far as these organisms are concerned, no species can be described directly from the host; consequently type specimens and type species based on these specimens become obsolete. Nor can the investigator depend upon the so-called natural conditions which for such a long time have been considered by the taxonomist as the only safe and sane conditions. In the laboratory one should

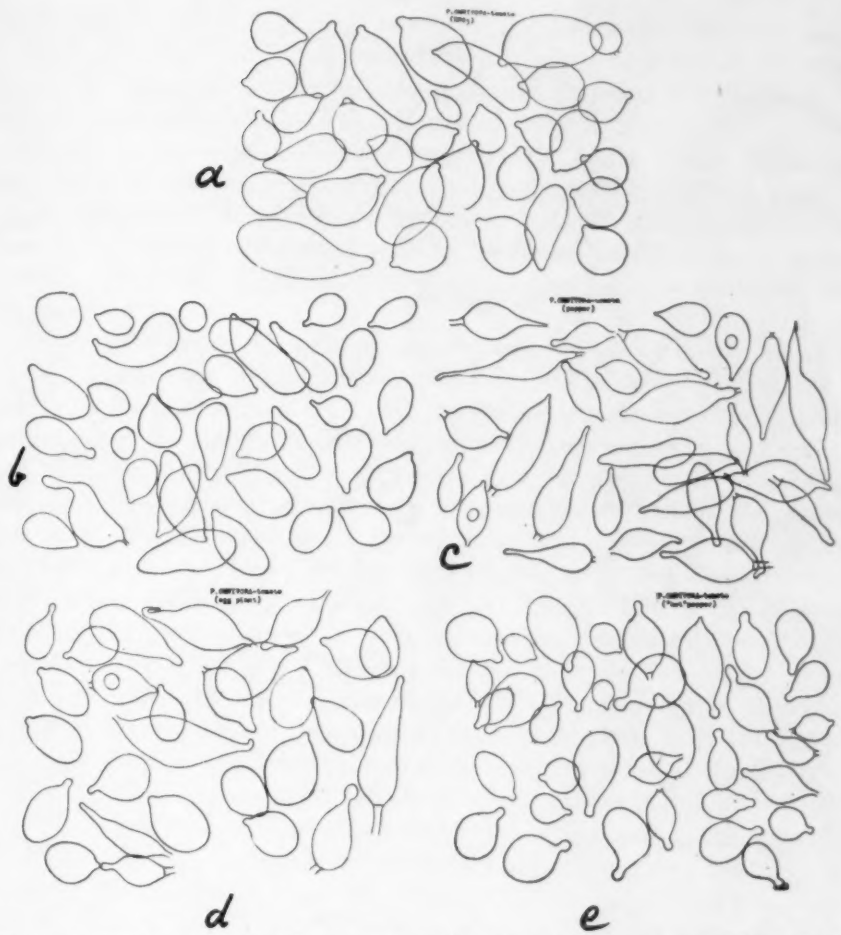

FIG. 5. Sporangia of *Phytophthora omnivora* (tomato strain). a, in potassium nitrate solution; b, on tomato; c, on bell-pepper; d, on egg-plant; e, on "hot" pepper.

be able to control conditions much better. It is true that no chemical compound is absolutely pure, that no temperature is always invariable, and no man-made technique infallible; but, on the other hand, temperature and moisture changes in nature are extremely variable, the competing microorganisms and their by-products are too complex and not only two different hosts but even two different parts of the same host are much more unlike than any two chemical compounds which may be used in the laboratory. It is true that, after the fungus begins to work upon a medium of known quality and quantity, an unknown substance may result, but the same thing applies to the natural conditions to a greater extent.

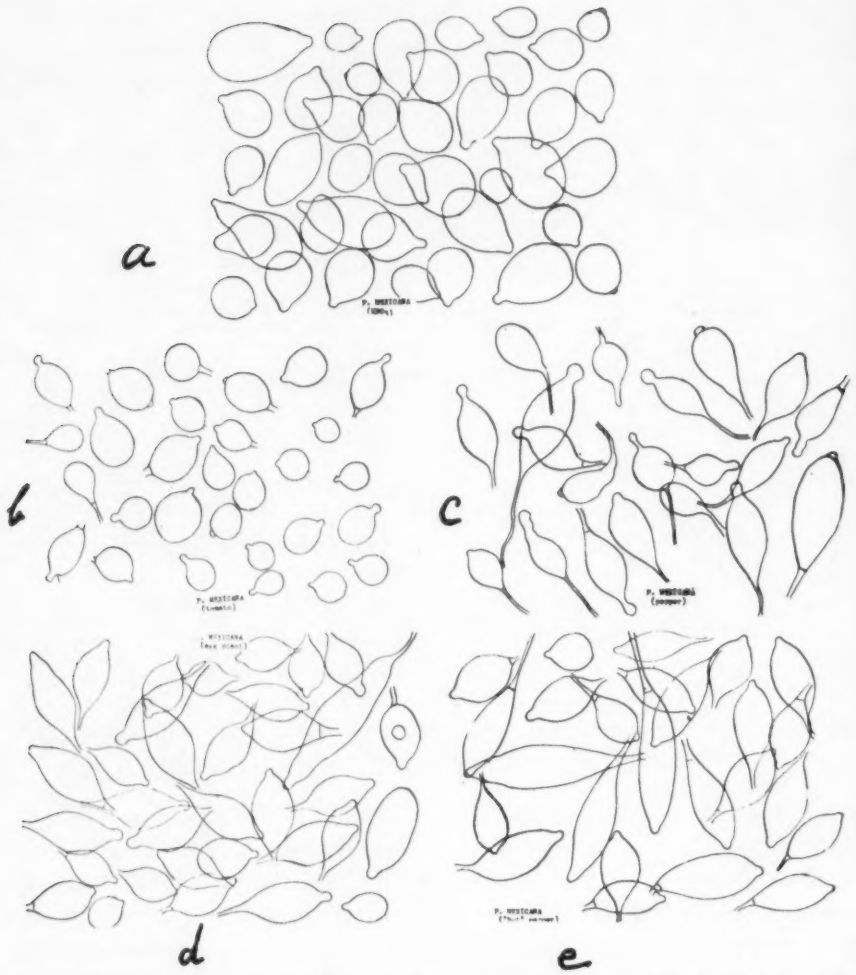

FIG. 6. Sporangia of *Phytophthora mexicana*. a, in potassium nitrate solution; b, on tomato; c, on bell-pepper; d, on egg-plant; e, on "hot" pepper.

If the final identification of a given *Phytophthora* is to be made in the laboratory, it is obvious that such complex media as oatmeal, cornmeal, prune juice and potato agars, which have been such great favorites of the plant pathologist, cannot be used. Nor can we consider the haphazard combination of chemicals known as Richard's solution and so commonly used whenever there is need of a synthetic solution. A simple solution of known quality and quantity should be preferred in all cases, and the conditions should be simplified still further by transferring a well-nourished mycelium to such a solution as M/100 potassium nitrate, which is known to stimulate

formation of sporangia. Such controlled conditions are infinitely more valuable than the haphazard methods which have been followed by many workers. This statement is based on some ten thousand measurements of the sporangia of 85 different Phytophthoras. However, no matter how carefully conditions are controlled and measurements made, a wide fluctuating margin should always be allowed because of the naturally plastic habit of these organisms. Measurements, therefore, together with other morphological data, can rarely possess sufficient merit to justify their use in the primary separation of species. Their cumulative value, however, can not be disregarded.

The writer wishes to make no generalizations concerning the effect of different hosts upon the morphology of fungi. Undoubtedly there are a large number of organisms which are not so plastic and can not be modified materially by a change of hosts. However, there are sufficient instances on record demonstrating that many other fungi can be made to alter their morphological features. Perhaps the most notable work along this line is that of Welles (7) on the genus *Cercospora*. Through many extensive experiments he found that the size and septations of conidiophores and conidia were modified by the environmental changes and by the effect of different hosts to such an extent that one species would readily pass for an entirely different species. Welles concludes that, so far as the genus *Cercospora* is concerned, morphological characteristics have no value. Taubenhaus (6) showed that, when sweet potato was inoculated with *Diplodia gossypii*, the fungus assumed the characteristics of the supposed genera of *Lasiodiplodia*, *Chaetodiplodia*, *Botryodiplodia* and *Diplodiella*. The same was found to be true when sweet potato was inoculated with *Lasiodiplodia*. A mere change of host plants, therefore, produced or abolished generic differences. The writer (1) demonstrated by controlled laboratory experiments that in the case of some fungi the presence or absence of stromata as well as pycnidial walls was controlled by food factors, thus altering the relationships of not only different genera but even the position of Sphaeropsidales and Melanconiales. There are examples in rust fungi where morphological characteristics are by no means constant or reliable. Long (3) observed that the influence of host plants upon the morphological characters of *Puccinia ellisiana* and *P. andropogonis* was very remarkable. *P. ellisiana* infects both *Viola* and *Pentstemon*, but when it passes through the latter host its characteristics entirely change and it becomes like *P. andropogonis*. When species of *Viola* were inoculated with *P. andropogonis*, the ensuing fungus became, so far as could be determined, *P. ellisiana*. Stakman and Levine (5) and Levine (2) state that congenial hosts do not affect different biological forms of *P. graminis*, whereas uncon-

genial hosts tend to decrease the size of uredinia and spores. Other adverse environmental conditions were also found to have similar effects.

The foregoing data clearly indicate that morphological characteristics, at least in many fungi, should not form the exclusive basis for the separation of species.

SUMMARY

1. A number of fleshy fruits and vegetables were inoculated with 85 different cultures of *Phytophthora*.

2. Nearly all of these organisms brought about a ready infection.

3. While the symptoms in all cases were much alike, there were remarkable modifications in the morphology of the sporangia due to the influence of different hosts.

4. It is concluded that identification of *Phytophthoras* directly from the host is misleading, and that controlled cultural work in the laboratory should always be used as a more trustworthy method of identification.

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FURTHER GERMINATION TESTS WITH TELIOSPORES OF RUSTS

W. E. Maneval

In an earlier paper (5) the writer reported the results of germination tests with the teliospores of ten species of rusts. It was pointed out that, although the teliospores of these rusts generally require a more or less definite resting period before germinating, a few teliospores may germinate in December or earlier, especially after prolonged floating on water or after alternate wetting and drying. During the past four years numerous additional tests with teliospores of six of these species of rusts (*Puccinia asparagi, P. helianthi, P. menthae, P. ruelliae, P. sorghi* and *P. windsoriae*) have given similar results, and germination of four species (*P. asparagi, P. menthae, P. sorghi* and *P. windsoriae*) has been obtained one to two months earlier than previously reported. The other four species have not been tested again.

Recently Mains (3) has reported that he obtained slight to abundant germination of teliospores of *Puccinia antirrhini* Diet. and Holw. as early as December or January. These results agree with those previously reported by Hockey (1), who obtained 14 to 22 per cent germination of the same rust, after exposure to cold or freezing, in January, 1921. Mains and Jackson (4) also succeeded in germinating teliospores of *Puccinia dispersa* Erikss. and Henn. from August to the following April and of *P. triticina* Erikss. from December to the next spring. Hoerner (2), although he used both fresh and overwintered teliospores of *P. coronata*, failed to obtain germination.

In addition to the ten species of rusts reported on in a previous paper (5), the writer has made germination tests of teliospores of the following rusts:

Melampsora medusae Thuem. on *Populus deltoides* Marsh.
Uromyces appendiculatus (Pers.) Link on *Phaseolus vulgaris* L.
U. aristidae Ell. and Ev. on *Aristida oligantha* Michx.
U. caryophyllinus (Schrank.) Wint. on *Dianthus caryophyllinus* L.
U. hedysari-paniculati (Schw.) Farl. on *Desmodium* sp.
U. howei Pk. on *Asclepias syriaca* L.
U. lespedezae-procumbentis (Schw.) Lagh. on *Lespedeza* sp.
U. spermacoces (Schw.) Thuem. on *Diodia teres* Walt.
Puccinia emiliae P. Henn. on *Calendula officinalis* L.
P. xanthii Schw. on *Xanthium* sp.

All collections of these rusts were made at or near Columbia, Missouri. The teliospores of the last two species require no rest period before germinating. All excepting *P. emiliae*, *P. xanthii* and *U. howei* are eu-types. Three of the eu-types, *Melampsora medusae*, *U. aristidae*, *U. caryophyllinus*, are heteroecious and the others autoecious.

The germination tests were all in duplicate and, unless otherwise stated, were made by floating the spores on distilled water and incubating at room temperature in alternate light and darkness (day and night). In the case of *Melampsora* and all species of *Uromyces* excepting *U. appendiculatus* and *U. caryophyllinus*, the number of germination tests has been small; however, teliospores of four species, *U. aristidae*, *U. hedysari-paniculati*, *U. howei* and *U. lespedezae-procumbentis*, germinated in two to six weeks.

TABLE 1.—*Germination of teliospores of eight different species of rusts*

Rust species	Date of collection	Date tested	Incubation period in days	Germination[a]
Melampsora medusae	Jan. 15, 1918	Jan. 28, 1918	2–3	++++[b]
Uromyces appendiculatus	Sept. 14, 1922	Sept. 16, 1922	28	+[c]
	do	do	60	++++
	Nov. 11, 1922	Nov. 11, 1922	2	+
	Oct. 12, 1926	Oct. 30, 1926	50	++++
U. hedysari-paniculati	Sept. 2, 1923	Sept. 3, 1923	40	+
	do	do	86	+++
U. howei	Oct. 7, 1923	Oct. 11, 1923	79	+
U. lespedezae-procumbentis	Oct. 2, 1923	Nov. 14, 1923	17	5 per cent
U. spermacoces	Dec. 14, 1924	Dec. 15, 1924	15	+
	do	Apr. 14, 1925	8	+[d]
	do	do	8	5–10 per cent[e]
U. aristidae	Oct. 13, 1925	Oct. 15, 1925	12	+
U. caryophyllinus	Nov. 1, 1925	Nov. 3, 1925	6	+
	Apr. 7, 1925	Apr. 7, 1925	15	+
	do	do	15	30 ± per cent[e]

[a] 0 = no germination; + = less than 1 per cent; ++ = 1–5 per cent; +++ = 10 ± per cent; ++++ = 30–90 per cent.

[b] Spores alternately wet and dry from Jan. 15 to Jan. 28.

[c] Spores kept during the winter as herbarium material at room temperature or lower germinated well in 1–2 weeks the following April.

[d] Spores kept as herbarium material at room temperature or lower from Dec. 14, 1924, to April 14, 1925.

[e] In 0.5 per cent KH_2PO_4.

Teliospores of *U. spermacoces* and of *Melampsora medusae* were collected in December and January respectively. Those of the former germinated in two to four days and of the latter, after alternate wetting and drying for two to three weeks, in two weeks. A few of the spores of *U. appendiculatus* collected in September, October or November in five different years (1922–1926) always germinated in one to four weeks. In table 1 are given the dates of collection and earliest germination, the incubation period, and certain other data regarding germination of teliospores of these eight rusts.

EFFECT OF LIGHT AND DARKNESS

At various times cultures of teliospores of *Puccinia emiliae* (Dec. 1, 1925; Dec. 21, 1925; Mar. 2, 1926) and of *P. xanthii* (Dec. 23, 1925; Mar. 2, 1926) were incubated in alternate light and darkness (day and night) and in continuous darkness. In all such tests a very high percentage of

TABLE 2.—*Effect of light and darkness on germination of teliospores of Uromyces caryophyllinus*

Series I

Date tested	Period in alternate light and darkness	Germination[a]	Period in darkness	Germination[a]
Dec. 1, 1925	Dec. 1 to Dec. 7	tr	Dec. 7 to Dec. 9	++++
do	Dec. 1 to Dec. 9	+	Dec. 9 to Dec. 11	++++
do	Dec. 1 to Dec. 11	0	Dec. 11 to Dec. 13	+++
Dec. 9, 1925	Dec. 9 to Dec. 10	tr	Dec. 10 to Dec. 13	++++
do	Dec. 9 to Dec. 11	tr	Dec. 11 to Dec. 15	++++
do	Dec. 9 to Dec. 12	tr	Dec. 12 to Dec. 15	++++
do	Dec. 9 to Dec. 14	tr	Dec. 14 to Dec. 16	++++
do	Dec. 9 to Dec. 18	tr	Dec. 18 to Dec. 20	++++
Dec. 21, 1925	Dec. 21 to Dec. 23	0	Dec. 23 to Dec. 26	++++
Nov. 28, 1925	Nov. 28 to Dec. 7	+	Dec. 7 to Dec. 9	++++
Oct. 30, 1926	Oct. 30 to Nov. 5	0	Nov. 5 to Nov. 8	100 per cent

Series II

Date tested	Period in darkness	Germination[a]	Period in alternate light and darkness	Germination[a]
Dec. 1, 1925	Dec. 1 to Dec. 7	0	Dec. 7 to Dec. 9	+
Dec. 21, 1925	Dec. 21 to Dec. 23	0	Dec. 23 to Dec. 26	2 ± per cent
Oct. 30, 1926	Oct. 30 to Nov. 5	20 per cent		
Dec. 9, 1925	Dec. 9 to Dec. 11	0	Dec. 11 to Dec. 16	tr[b]
do	Dec. 9 to Dec. 13	0	Dec. 13 to Dec. 16	tr[b]
do	Dec. 9 to Dec. 16	0	Dec. 16 to Dec. 18	0[b]

[a] 0 = no germination; tr = trace; + = less than 1 per cent; ++ = 1–5 per cent; +++ = 10 ± per cent; ++++ = 30–90 per cent.

[b] Germination ++++ after being replaced in darkness for 2 days.

spores germinated in 2 to 3 days under the former conditions but very few in the dark. Similar results were obtained with spores of *P. helianthi* (Dec. 1, 1925; Dec. 9, 1925; Feb. 25, 1926; Apr. 24, 1926), many cultures having been tested. Moreover, with all three kinds of rusts, darkness proved less favorable than alternate light and darkness for the production of sporidia. A very large number of germination tests with teliospores of *U. caryophyllinus* was made. Generally a few spores germinated in alternate light and darkness in 6 to 8 days and a large number in 3 to 4 weeks; but sometimes they germinated better in continuous darkness. However, if, after the cultures had been kept in alternate light and darkness for 2 to 9 days, they were placed in continuous darkness, a high percentage (50 ±) of spores always germinated in two days. Cultures kept in continuous darkness for a week generally failed to germinate; but if they were then kept in alternate light and darkness for two days and replaced in the dark, a very large proportion of spores germinated in two more days. Some results of these tests are presented in table 2.

OVERWINTERING OF TELIOSPORES OF PUCCINIA EMILIAE AND OF PUCCINIA XANTHII

In November, 1925, *Puccinia emiliae* was found in considerable abundance on calendulas in the greenhouses of the Department of Horticulture at the University of Missouri. It is not known how the rust was introduced. The calendulas were grown from seeds. This is apparently the first record of the occurrence of this rust in Missouri, although it had previously been reported from Indiana, Illinois, Kansas and Nebraska. Since teliospores of *P. emiliae* and of *P. xanthii* require no rest period before germinating, tests were made at intervals to determine how long they would continue to germinate. Collections of *P. emiliae* (Nov. 17, Nov. 27, and Dec. 1, 1925) and of *P. xanthii* (Oct. 1, 1925) were kept in a room that was cool during the winter but quite warm part of the time after May. Spores of *P. emiliae* were also kept in an ice chest. In all tests excepting the first, pieces of host tissue bearing spores were floated on distilled water. The results are given in table 3.

It is evident that teliospores of these two rusts may live over winter or longer under varying conditions of temperature and humidity as well as, in the case of *P. emiliae*, under rather uniform conditions in an ice chest. Taubenhaus (6) collected leaves of hollyhock bearing teliospores of *Puccinia malvacearum* November 26, 1908, and kept them in wire baskets at a low temperature both indoors and outdoors. The teliospores germinated from December 2 until April 10. He concludes that this rust may hibernate as teliospores on seeds. It is not impossible that *P. emiliae* may have been introduced into the greenhouses at Columbia as teliospores on seeds. It

TABLE 3.—*The viability of teliospores of Puccinia emiliae and Puccinia xanthii as measured by germination tests*

Rust species	Date of collections, 1925	Dates tested	Germination[a]
Puccinia emiliae	Nov. 17; kept in ice chest	Dec. 19, 1925; Jan. 13, Feb. 10, Mar. 2, Apr. 5, May 3, May 22, 1926	++++
		June 7, 1926	+++
		Oct. 1, 1926	++
do	Nov. 17	Dec. 19, 1925; Jan. 13, Feb. 10, Mar. 2, Apr. 5, May 3, May 22, June 7, 1926	++++
do	Nov. 27	Dec. 19, 1925; Jan. 13, Feb. 10, Mar. 2, Apr. 5, May 3, May 22, 1926	++++
		June 7, 1926	+
do	Dec. 1	Dec. 19, 1925; Jan. 13, Feb. 10, Mar. 2, Apr. 5, May 3, May 22, 1926	++++
		June 7, 1926	+++
		Oct. 1, 1926	+
Puccinia xanthii	Oct. 1	Oct. 1, Nov. 27, Dec. 19, 1925; Jan. 13, Feb. 10, Feb. 23, Mar. 2, Apr. 5, May 3, May 22, 1926	++++
		June 7, 1926	+++
		Oct. 1, 1926	0

[a] 0= no germination; + = less than 1 per cent; ++ = 1–5 per cent; +++ = 10 ± per cent; ++++ = 30 to 90 per cent.

disappeared in our greenhouses about midwinter but developed again in October, 1926.

EFFECT OF POTASSIUM DIHYDROGEN PHOSPHATE ON GERMINATION

In various tests, teliospores of certain rusts germinated better in a solution of KH_2PO_4 than in distilled water. Spores of *Uromyces spermacoces* collected December 14, 1924, were tested April 14, 1925. After eight days only a few spores had germinated in distilled water, but 5 to 10 per cent had germinated in 0.5 per cent KH_2PO_4 (table 1). In a test with spores of *U. caryophyllinus* April 7, 1925, in alternate light and darkness, after fifteen days only a few had germinated in distilled water but 20 to 50 per cent

had germinated in 0.5 per cent KH_2PO_4 (table 1). Similar results had previously been reported (5) for spores of *Puccinia helianthi* and have been obtained repeatedly since in solutions of 0.2 to 0.5 per cent KH_2PO_4 and in distilled water.

GERMINATION OF TELIOSPORES OF PUCCINIA HELIANTHI IN SOLUTIONS OF VARIOUS CHEMICALS

Germination of teliospores of sunflower rust was tested in solutions of hydrogen peroxide, chloroform, ether, dextrose, maltose, sucrose, ammonium nitrate, potassium nitrate, calcium nitrate, potassium permanganate, sodium chloride and combinations of some of these. Of course with organic compounds such as sugars, on account of the development of contaminating organisms, it was necessary to use spores that would germinate freely in a few hours. Spores germinated normally, apparently, in concentrations as high as 0.1 per cent NH_4NO_3, 0.25 per cent KNO_3, 0.125 per cent NaCl, 1.0 per cent $Ca(NO_3)_2$, 0.5 per cent KH_2PO_4, 1.0 per cent maltose and sucrose and 2.0 per cent dextrose; but more or less abnormally in higher concentrations of these chemicals. Teliospores floated on distilled water to which 1 or 2 drops of chloroform or ether per 10 cc. of water had been added germinated about as well as in distilled water. Larger quantities of these chemicals partially or completely inhibited germination. Likewise, exposure of wet spores on leaves to vapor of ether (1–15 drops to 50 cubic inches of space) for 1 to 48 hours did not improve germination. When hydrogen peroxide was added to cultures (2 drops to 10 cc. of water), spores sometimes germinated more readily than in water, but these results could not be consistently duplicated. Pre-soaking spores for 24 hours in a 1 or 2 per cent solution of $KMnO_4$ did not increase the percentage of germination over that in the checks. It is evident therefore that, with the exception of KH_2PO_4, the chemicals tested had little or no favorable effect on germination.

TELIOSPORES THAT FAILED TO GERMINATE

Attempts to germinate the teliospores of the following rusts have been unsuccessful, in some cases probably because the incubation period was too short. For the first five species this period was approximately one month and for the last three approximately two months. The months when the tests were started are indicated after each species, and in all cases the spores were of the current season.

Puccinia amorphae Curt. on *Amorpha fruticosa* L. Oct. and Jan.
P. bullata (Pers.) Wint. on *Taenidia integerrima* (L.) Drude May
P. coronata Corda on *Avena sativa* L. Jan.

P. osmorhizae (Peck) Cke. and Pk. on *Osmorhiza longistylis* (Torr.) DC.	May
P. podophylli Schw. on *Podophyllum peltatum* L.	May
P. bardanae Corda on *Arctium minus* Bernh.	Oct.
P. graminis Pers. on *Triticum vulgare* Vill.	Oct.
P. pruni-spinosae Pers. on *Prunus persica* (L.) Stokes	Nov.
and on *Prunus serotina* Ehrh.	Oct.

SUMMARY

The principal results of these and my previous observations on germination of teliospores of nearly thirty species of rusts may be summarized as follows:

1. If spores are kept alternately wet and dry, or floated continuously on distilled water at room temperature in alternate light and darkness, some generally germinate in 2 to 6 weeks.

2. As the season advances there is a decrease in the time necessary for germination to begin and for complete germination (highest possible percentage).

3. Treatment with various chemicals, except to adjust the H-ion concentration, had little or no favorable effect. However, spores may germinate in relatively high concentrations of certain chemicals.

4. Spores of *Puccinia helianthi, P. emiliae* and *P. xanthii* germinated more readily and produced many more sporidia in alternate light and darkness than in continuous darkness.

5. Spores of *Uromyces caryophyllinus* germinated very readily if cultures were kept in alternate light and darkness for two to several days and were then placed in continuous darkness. Even in continuous darkness germination sometimes occurred, and in such cases in less time and more profusely than in alternate light and darkness.

6. Spores of some rusts formed late in the growing season apparently germinate more readily than those formed earlier.

7. Under certain conditions spores of *Puccinia emiliae* and of *P. xanthii*, although they require no rest period, may continue to germinate after being stored over winter or longer (8 to 10 months).

8. Attempts to germinate the teliospores of several species of rusts, collected soon after spore formation, have never been successful, in some cases probably because the incubation period has been too short.

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SOME CHEMICAL TREATMENTS OF SOIL FOR THE CONTROL OF DAMPING-OFF FUNGI

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Seedlings grown in soils treated with steam or formaldehyde are without protection against subsequent introduction of pathogenic fungi. The expense and other factors render these methods of treatment slow of adoption. There is therefore an urgent need for fungicides effective in concentrations that may safely be used in association with the underground parts of growing plants and that may afford continuous protection.

It is apparent that damping-off cannot be approached as a general problem, since marked specificity of pathogenes for suscepts and of chemicals for both pathogenes and suscepts is well known. This paper is concerned chiefly with the damping-off of tomatoes and cabbage in the greenhouse.

PREVIOUS WORK

Although some success was reported in the chemical treatment of soils by early workers, among them Halsted (9) in 1900, it is in the last decade that more definite progress in this direction has been made. Bewley (3) has made extensive tests of copper compounds and other chemicals in the soils of glasshouses. His Cheshunt mixture (2) proved effective in the control of damping-off of tomato and other plants when both *Phytophthora* and *Rhizoctonia* were apparently involved. Gratz (8) tested this mixture for *Rhizoctonia* of cabbage with discouraging results. Sherbakoff (14) used copper sulfate for *Rhizoctonia* of lettuce with some success. Gloyer and Glasgow (7) and Clayton (4) have successfully used mercury compounds for rhizoctonia, damping-off, and other diseases of crucifers. Monteith and Harmon (13) found mercurous chloride to be the most satisfactory of many chemicals tested in the control of brown patch of turf caused by *Rhizoctonia* spp. Major (12) found that tobacco plants were injured by mercury compounds when applied in sufficient concentration to control Thielavia damping-off. Hartley (10) and Wiant (15) have successfully employed sulfuric acid, aluminum sulfate, and various compounds of mercury for the control of damping-off of coniferous seedlings. Much has been written concerning the beneficial effect of chemicals upon seedlings aside from disease control. It seems probable that a considerable part of this apparent stimulation may be accounted for, however, by the control of pathogenic fungi (1).

MATERIALS AND METHODS

The fungi employed in these experiments were two strains of *Phytophthora*, isolated from tomato; and three strains of *Corticium vagum*

B. and C., isolated from cabbage, beet, and pepper. The soil was a rather heavy clay loam used mostly in flats approximately 18 x 18 x 2 inches. Inoculations were made in most cases from cultures of the fungus grown on steamed wheat kernels. Occasionally, infested soil was used. A known quantity of seed (500 seeds per flat in nearly all cases) was used. Unless otherwise indicated, treatments were made immediately after planting. The chief chemicals employed were copper carbonate, copper sulfate, mercuric chloride, and chlorophenol mercury.

EXPERIMENTS WITH TOMATOES

In several preliminary trials with tomatoes, the Cheshunt mixture (2) and copper carbonate gave similar and substantial increases in total and disease-free seedlings on soil inoculated with *Phytophthora*. In subsequent tests, copper carbonate was used alone or in comparison with mercury compounds, and occasionally with other chemicals.

Treatments at planting time.—In table 1 are summarized the results of treating tomatoes at the time of planting on soil inoculated with *Phytophthora*. The varieties Ponderosa and John Baer were used. *Rhizoctonia* from cabbage and *Botrytis* from lettuce did not influence the stand of tomato seedlings.

TABLE 1.—*The effect of treating tomatoes at planting time on Phytophthora-infested soil*

Treatment	No. plants surviving in 7 experiments[a]						
	1	2	3	4	5	6	7
Copper carbonate 2 gm.	371						
do 3 gm.		357					
do 5 gm.			214			302	269
			292			274	284
Corona copper carbonate 15 gm.			221	224	224		
			223	282	237		
Colloidal copper							316
$CuSO_4$ 3 gm.–1000 cc. H_2O							229
$HgCl_2$ 0.5 gm.–1000 cc. H_2O						364	
$HgCl_2$ 0.5 gm.– 800 cc. H_2O							244
$HgCl_2$ 1 gm.–1600 cc. H_2O							115
Uspulun 2 gm.–1000 cc. H_2O						338	
Uspulun 2 gm.– 800 cc. H_2O							273
Uspulun 4 gm.–1600 cc. H_2O							138
Check	227	102	145	91	65	334	302
Check			136	101	67	215	193

[a] Each number represents the plants produced in a flat planted with 500 seeds.

The duration of the experiments was variable, extending over periods as long as 47 and 49 days from the time of planting to the taking of final notes. Corona copper carbonate with copper content approximately equivalent to five grams of undiluted copper carbonate was used, and gave essentially similar results. Strengths at least double those employed here have been used on tomato without appreciable injury. For convenience in application, the insoluble carbonates, as well as the soluble chemicals, were applied in water. The average number of surviving plants in all copper carbonate flats was 269.5, and in the corresponding checks 164.8, out of a total of 500 seeds sown. While the higher concentrations of the mercury compounds caused obvious injury, the lower concentrations offer considerable promise for further testing.

Treatments after planting time.—It is obvious that a method of controlling damping-off involving treatments begun after the disease appears among the seedlings is very desirable. Four experiments were made with this possibility in mind. No marked or consistent benefit was derived from applications of copper carbonate made after damping-off had appeared. In one case an application made six days after planting was quite as effective as were those made at planting time, but no disease was noted in these flats until nine days after planting. It seems probable, however, that late treatments might be beneficial to large areas in which localized infestations appear.

Experiments with tomatoes on soil inoculated with Rhizoctonia.—In several earlier experiments, the *Rhizoctonia* from cabbage produced no appreciable damping-off of tomatoes. Later, in a single experiment, *Rhizoctonia* from beet produced only slight injury. Still later a strain isolated from pepper seedlings was tested on pepper, tomato, and cabbage in comparison with the cabbage strain. The pepper strain was pathogenic to pepper and tomato but not to cabbage. The cabbage strain produced severe damping-off on cabbage and little or no disease on pepper or tomato. Tomatoes were then planted in four flats inoculated with the pepper strain, and three of these were treated respectively with mercuric chloride one-half gram, mercuric chloride one gram, and Uspulun two grams, each in one liter of water. The fourth served as a check. Final counts on these flats were respectively 329, 68 (chemical injury), 395, and 88. In another similar experiment of eight flats, copper carbonate (10 grams) and copper sulfate (3 and 6 grams) produced as good stands as the weaker mercury compounds, but the loss in the check was much less severe. The stronger mercuric chloride (1 gram) and Uspulun (4 grams) again caused serious injury.

Again 10 flats were similarly treated, with the following results: mercuric chloride (0.5 gram), 371 and 341; copper sulfate (6 grams), 97 and

115; copper carbonate (5 grams), 95 and 181; Corona colloidal copper (not measured), 25 and 54; checks, 13 and 30 plants per flat. Copper sulfate caused marked retardation of the seedlings in this experiment but did not prevent serious damping-off. It is apparent that under these conditions of severe damping-off the copper compounds are not effective in controlling *Rhizoctonia*.

Distribution of chemicals in soil.—It has been shown that copper compounds are very quickly taken out of suspension or solution when added to soils (5, 11). It seemed, therefore, that these might profitably be introduced into the soil to greater depth than would be reached by the methods employed in the earlier part of this work. A one per cent solution of sodium chloride with a one-half per cent solution of copper sulfate was first used (one liter per flat), in four flats inoculated with *Phytophthora*. Although the concentration and quantity of chemicals was much lower than those used by Hunt and his colleagues (11), there was a marked retardation in germination and early development of the seedlings. These flats, however, produced substantially greater numbers of seedlings than untreated flats. The same concentration of sodium chloride was then tested alone and in combination with Corona copper carbonate, 15 grams per flat, on Phytophthora-inoculated flats. Emergence of seedlings was retarded one to two days by salt alone, and four days by salt plus copper carbonate. Total seedling counts were highest on the copper carbonate flats, lowest on the salt flats, and intermediate on untreated flats (averages 330, 272, and 293 respectively).

In two experiments copper carbonate at double the quantity generally used for surface treatments was thoroughly mixed with the soil by means of a barrel churn. In both instances following inoculation there were only small increases in numbers of seedlings over those on untreated soil. In one of these, in flats given the usual surface treatment, there were twice as many plants as in the checks. It seems, therefore, that under these conditions the concentration of chemicals near the surface of the soil is more desirable than vertical distribution through the soil.

Chemical injury to tomatoes.—Copper carbonate, when used alone in the quantities usually employed here, has not produced injury whether applied at planting time or on the growing plants, with one exception, in which the plants were somewhat retarded. Heavier applications have occasionally caused a gradual yellowing of plants after several weeks. Copper sulfate at six grams per flat sometimes caused considerable retardation in growth of seedlings.

The tomato is more sensitive to injury by mercury compounds than is cabbage. Applications of these chemicals at planting time may either reduce the percentage of germination or merely retard the rate of growth of

seedlings. Mercuric chloride (1–1600) or Uspulun (1–400) applied to the growing plants kill areas on the foliage and injure the stems; the injury is most pronounced slightly above the ground level. Injury in these experiments has usually been more marked with mercuric chloride than with Uspulun when the materials were used in the proportions given above. Since this is the only appreciable difference noted between these chemicals, the figures from five trials bearing on this point are assembled in table 2. The soil had been inoculated with a damping-off fungus in all of these cases except one, but the consistently high counts of seedlings in flats in which lower concentrations of chemicals were used are sufficient practically to eliminate damping-off from consideration in comparing the results of heavier applications. It is believed that variations in the temperatures of the greenhouses and of the solutions employed has an important bearing on the amount of injury produced. Gassner (6) has shown that even with seed treated at the same temperature, a marked variation in injury follows when the seeds are germinated at different temperatures.

TABLE 2.—*The effect of mercuric chloride and Uspulun on stands of tomato seedlings when applied at planting time*

Treatments	No. plants surviving in 5 experiments[a]				
	1	2	3	4	5
$HgCl_2$ – 0.5 gm. – 1000 cc. H_2O		315	329		364
do – 800 do	244				
$HgCl_2$ – 1 gm. – 1600 do	115				
do – 1000 do		74	68	373	
Uspulun – 2 gm. – 1000 do		314	395		338
do – 800 do	273				
Uspulun – 4 gm. – 1600 do	138				
do – 1000 do		196		406	

[a] Each number represents the plants produced in a flat planted with 500 seed.

EXPERIMENTS WITH RHIZOCTONIA OF CABBAGE

Since *Rhizoctonia* in the soil is one of the fungi especially resistant to chemical treatment, several experiments were made in the control of this fungus with cabbage as the test plant. The results of seven such tests are shown in table 3. All figures included here represent results in flats except those in the last column, which were obtained in a greenhouse bed. The Corona colloidal copper was peppered rather heavily on wet soil and was not measured. In general, mercuric chloride and Uspulun are effective in the control of this fungus; whereas the copper compounds are relatively ineffective. At times, however, in treated flats, "wire stem" (8) appeared

and some seedlings were dead before the final counts were made, indicating that a second application would be necessary in some cases. Delayed single applications, as in the tests on tomatoes, were not effective. The slight injury caused occasionally by mercury compounds was not greater in the case of corrosive sublimate than that caused by chlorophenol-mercury.

TABLE 3.—*The effect of treating cabbage at planting time on Rhizoctonia infested-soil*

Treatment	No. seedlings surviving in 7 experiments[a]						
	1	2	3	4	5	6	7
$HgCl_2$ – 0.5 gm. – 1000 cc. H_2O				296	254		
do 12 days after planting					146		
$HgCl_2$ – 1 gm. – 1000 cc. H_2O					301		
do – 1600 do						216	
Uspulun – 2 gm. – 1000 do				346	240		
do 12 days after planting					160		
Uspulun – 2 gm. – 800 cc. H_2O			221				
do – 3 gm. – 1000 do	260						
do – 4 gm. – 1600 do						222	
do – 4 gm. – 1000 do					268		
do – 4 gm. – 800 do			273				
do – 5 gm. – 1000 do 37 days before planting		169					
do – 2.6 gm. per sq. ft.							344
Copper carbonate – 1 gm. per sq. ft.							17
Corona copper carbonate – 30 gm.			34				
Colloidal copper Corona						40	
Colloidal copper – 2 gm. – 1000 cc. H_2O			9				
Colloidal copper – 4 gm. – 1000 cc. H_2O			12				
$CuSO_4$ (crystal) – 3 gm. 1000 cc. H_2O					171		
do – 6 gm. do					241		
Check	34	51	0	111	181	8	1
Check					165		

[a] Each count represents the seedlings produced from 500 seeds.

OTHER OBSERVATIONS

The dictum that "all general statements are in error including this one" finds ready application in the consideration of damping-off diseases, the problems of which are multiplied by each kind of soil, pathogene, suscept, and chemical involved.

The conditions imposed here were, at least in many cases, more severe than those of the usual greenhouse practice. In some experiments a considerable number of the seedlings of check flats never appeared above ground. Thus a phenomenal difference in total seedlings may be recorded

for treated and untreated flats. It has been recognized more recently that this condition has been mistaken for chemical stimulation (1).

A marked difference is noted in these experiments in the action of different chemicals upon various plants as well as upon the different fungi. For example, copper carbonate was effective in controlling *Phytophthora* of tomato and apparently *Botrytis* sp. of lettuce, but almost completely ineffective in control of *Rhizoctonia* of cabbage. The same material caused no injury to cabbage, tomato, cucumber, lettuce and beets, but was found by Wiant (15) to cause severe injury to conifers. Uspulun used in concentrations that caused no apparent injury to cabbage was injurious to bean, cucumber, tomato, and lettuce. Tobacco appears to be more sensitive to both copper and mercury compounds in the soil than was any other plant studied.

In several experiments, plantings of tomato and cabbage were treated with copper and mercury compounds with the primary purpose of studying chemical stimulation of the seedlings. Neither in these nor in other experiments has there been consistent evidence of increase in size of plants on treated flats. Increases in numbers of seedlings, whenever this occurred, is more reasonably explainable on the basis of disease control.

SUMMARY

Copper carbonate, mercuric chloride, and Uspulun controlled damping-off of tomatoes caused by *Phytophthora* spp.

The mercury compounds caused injury to tomatoes in concentrations which did not injure cabbage.

The mercury compounds were effective in controlling *Rhizoctonia* in cabbage and tomato plantings; whereas copper carbonate and two forms of colloidal copper were almost completely ineffective.

Treatments after damping-off had appeared were of little value to tomatoes or cabbage.

There was no evidence of chemical stimulation in any of these experiments.

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A BACTERIAL DISEASE OF BOWLESIA

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Bowlesia septentrionalis Coulter and Rose is an annual caulescent herb belonging to the Ammiaceae. It occurs in rich soil or shaded places throughout the Southwest from Texas to California. Plants begin to appear during December, reach the maximum abundance during April and May, and disappear as the hot, dry weather approaches.

During several seasons a bacterial disease has been noted frequently on plants growing in the vicinity of Austin, Texas. Symptoms appear on the leaf blades and petioles shortly after the leaves unfold and, as the growing season advances, the infection becomes so general that uninfected plants are rare. The diseased leaves show numerous characteristic water-soaked spots which very soon become almost black. These spots are frequently marginal but not always so. With age they become somewhat irregular in shape and the tissue drys out, becoming reddish brown. When the petiole is infected, the entire leaf droops and withers. It often happens that entire lobes of the leaf are killed (Fig. 1). The youngest spots have the appearance of a bacterial disease. That they are caused by one of the green fluorescent species of plant-pathogenic bacteria has been proved by isolation of the organism and subsequent inoculation of healthy plants with pure cultures.

For purposes of isolation, young spots from the first infected leaves which appeared in January were chosen. The leaves were rinsed through several changes of sterile water but were not disinfected. The spots were removed with sterile instruments and thoroughly crushed in a few drops of sterile broth. A single loopful of this inoculum was then spread over the surface of an agar plate by means of the loop needle. After 24 hours incubation at 27° C. the colonies are visible but quite small. They increase in size and reach the maximum after about 48 hours incubation. On agar plates prepared in the above manner numerous colonies develop. In all of the plates prepared for isolation there was a single predominating type of colony. Occasional contaminations with soil bacteria occur, but such colonies are invariably few in number and not easily mistaken for the true causal organism. Single, well isolated colonies were transferred to plain agar slants. The cultures so established were subsequently studied as to cultural reactions, morphology, and physiological and pathogenic properties.

CULTURAL FEATURES

Cultures in all media except gelatin were incubated at 27° C. The reaction of the culture media was adjusted to pH 7 unless otherwise stated.

Agar Plates. On the surface of agar plates the colonies reach a diameter of 5–7 millimeters. They are smooth, moist and glistening, somewhat yellowish with entire margin. On beef infusion agar a beautiful greenish pigment is produced which diffuses throughout the agar. The growth is quite viscid when touched with the needle.

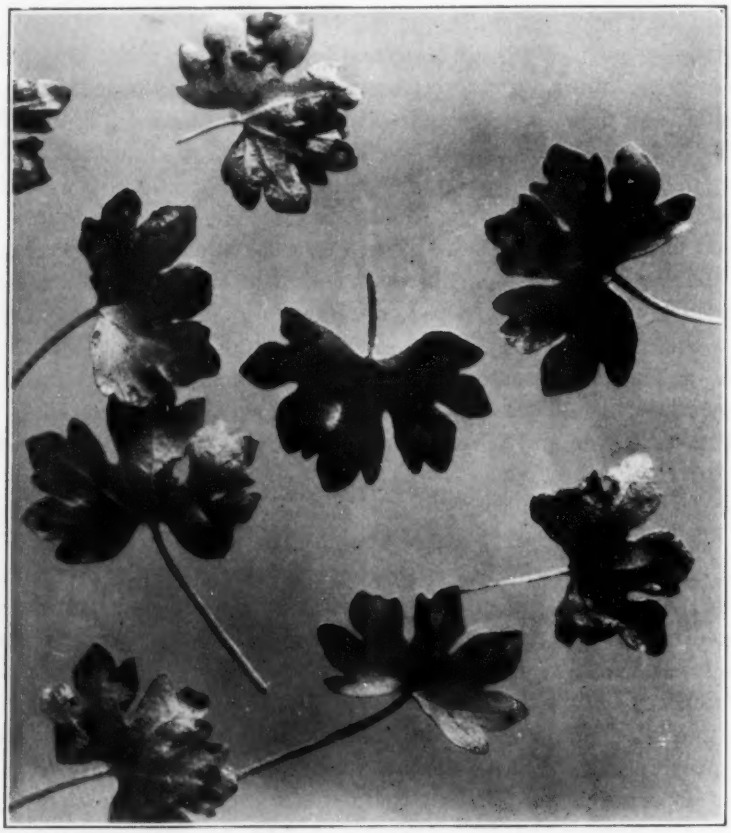

FIG. 1. Bacterial spots on leaves of *Bowlesia septentrionalis.*

Agar Slant. On the sloped surface of agar inoculated with a straight needle the growth becomes evident as a fine delicate line at the end of 24 hours. In 48-hour cultures the growth has attained a width of 2–4 mm. with smooth or slightly undulate margin. It is yellowish, moist, glistening

and viscid. The green fluorescent pigment is produced abundantly on infusion agar. After a few days the growth changes from a copious viscid mass to a thin film. This feature is quite characteristic of all the agar cultures.

Beef Infusion Broth. In young cultures the broth is uniformly cloudy throughout the medium. As the culture becomes older, a heavy viscid sediment forms at the bottom while small zoogloeal masses occur at the surface. The broth becomes greenish in cultures five days old.

Gelatin. The liquefaction of gelatin occurs promptly. In cultures 48 hours old there is a napiform area of liquefaction. By the end of seven days the process of liquefaction is completed.

Steamed Potato. The organism grows well on the sloped surface of steamed potatoes. In old cultures there is a heavy yellow slime covering the entire surface.

Steamed Sweet Potato. Growth is copious on this medium. It is more yellowish than growth on agar.

Litmus Milk. Coagulation of milk occurs in 48 hours. The reaction becomes alkaline. Digestion of the protein begins after the second day and progresses rather slowly. In cultures seven days old there is a heavy viscid mass at the bottom with some solid particles floating at the surface. The litmus is not reduced.

Coagulated Blood Serum. Loeffler's blood serum medium becomes covered with a slimy yellowish growth. Liquefaction occurs slowly.

Crowe's Medium. On a medium described by Crowe consisting of one part glucose agar and three parts of defibrinated blood the growth is moderately heavy with a greenish color.

Sugar Agars. On plain extract agar containing 1 per cent glucose, sucrose, maltose or lactose and 1 per cent of Andrades indicator the growth is best at the top but extends along the line of inoculation to the bottom of the tube. Acid is produced from glucose and maltose.

Cohn's Solution. Growth occurs in Cohn's solution producing a uniform turbidity. The growth is not, however, abundant.

Fermi's Solution. Growth is similar to that on Cohn's medium.

Uschinsky's Solution. The growth begins promptly and with much greater vigor than in the other synthetic media.

Ferric Ammonium Citrate Agar. On the sloped surface of synthetic agar containing 0.8 per cent of the citrate as the sole source of carbon, growth is vigorous. A rusty red deposit of iron hydroxide occurs on the surface, indicating that the citrate is utilized.

MORPHOLOGY

The organism is rod-like, straight with rounded ends, borne singly or sometimes in pairs and short chains. It stains readily with aqueous solu-

tions of gentian violet, fuchsin or methylene blue. When stained by Gram's method the gentian violet is not retained. Capsules have not been demonstrated. Cultures grown on a variety of media were stained by both the Hiss and Ribbert's methods. Young cultures are very actively motile by means of bipolar flagella. The flagella have been stained by means of Loeffler's, Casares-Gil's and Gray's methods. They occur only at the poles and are generally two or three in number at each end of the cell. They are slender, flexuous, about three times the length of the rod. Spores have not been shown either in stained or unstained mounts. Involution forms, if present, are never prominent. In fresh young cultures grown on beef infusion agar and stained with aqueous gentian violet, the rods measure 1.2 to 1.6 microns in length by 0.5 to 0.7 microns in diameter. Color granules have not been observed.

PHYSIOLOGICAL CHARACTERISTICS

Thermal Relations. The optimum temperature for growth is about 27° C. Growth does not occur at 37° C. nor at −1° C. Below 5° C. development takes place very slowly. That the organism is capable of developing at rather low temperatures is evident from the fact that the disease is widely spread during the cool winter months when the temperature varies from about 20 to 75° F. The thermal death point for 48-hour cultures in neutral broth is 49° C.

Resistance to Desiccation. The ability to survive desiccation was tested by placing drops of a 48-hour broth culture on sterile cover slips and transferring one of these to sterile broth at daily intervals until no growth was obtained. Under this condition, the organism has little resistance to desiccation, as growth failed at the end of four days. It is evident, however, that under natural conditions it must be able to survive much longer in the dry summer months.

Oxygen Relations. In fermentation tubes containing various sugars or other fermentable carbon compounds, growth occurs only in the open arm of the tube. Anaerobic cultures fail to grow. The technique used was that of oxygen absorption by means of pyrogallic acid and potassium hydroxide.

Relation to Reaction of Medium. The organism grows through a rather wide range of H-ion concentration. The limits for growth on plain extract peptone agar appear to be about pH 4.5 and 8.6 with the optimum growth at pH 7.2. In 1 per cent peptone beef extract broth the reaction was changed from an initial pH 7.1 to pH 8.6.

Fermentation. The ability to ferment various carbon compounds was tested in neutral 1 per cent peptone beef extract broth plus brom thymol blue indicator. Acid, but no gas, was produced in glucose, maltose and xylose. No fermentation occurred in sucrose, lactose, arabinose, mannite,

galactose, levulose, cellobiose or inulin. The experiment was continued for 24 days. At the end of this time the xylose and maltose broth had become alkaline, although the glucose remained acid.

Indol Production. Cultures were grown seven days in tryptophane broth and tested for indol by Ehrlich's method. Indol is produced.

Production of Hydrogen Sulphide. The test for hydrogen sulphide was made by culturing the organism in beef extract agar plus lead acetate. A blackening of the agar occurred promptly, indicating the presence of hydrogen sulphide.

Reduction of Nitrates. Cultures were prepared in nitrate broth and tested for nitrites by the sulphanilic acid and naphthylamine hydrochloride test. Nitrates are reduced to nitrites.

Ammonia Production. Cultures grown in beef extract peptone broth and tested with Nessler's reagent show the presence of ammonia.

Reduction of Methylene Blue. The dye is reduced in peptone broth cultures.

BIOLOGICAL RELATIONS

The organism is pathogenic to *Bowlesia septentrionalis.* Plants sprayed with a water suspension prepared from an agar culture and kept in a moist atmosphere produced typical spots within five days. Leaves which were punctured with a needle and inoculated by placing a small bit of an agar culture in the wounded tissue developed enlarged spots which contrasted sharply with uninoculated punctures. Similar methods of inoculation on other plants gave no positive results. Other species growing near *Bowlesia* in the field are not infected so far as is known.

TECHNICAL DESCRIPTION

This organism does not appear to agree in all particulars with any of the yellow fluorescent plant pathogens which have been described. It is therefore considered as a new species in the genus *Phytomonas.*

Phytomonas bowlesii n. sp.

A short rod with rounded ends, borne singly, in pairs or very short chains; actively motile by means of bipolar flagella which are two to five in number at each end of the rod; no spores or capsules distinguished; produces green fluorescent pigment; liquefies gelatin and blood serum; coagulates milk and digests the casein; does not reduce litmus; reduces methylene blue and nitrates; produces hydrogen sulphide, ammonia, and indol; grows in Cohn's, Uschinsky's and Fermi's solutions; utilizes ferric ammonium citrate and deposits iron hydroxide; optimum temperature 27° C., minimum

temperature −1° C., maximum temperature 35° C.; thermal death point 49° C.; resists desiccation four days; stains with basic analine dyes; not acid fast, negative to Gram's method of staining; optimum reaction of medium pH 7.2, minimum pH 4.5, maximum pH 8.6, pathogenic to *Bowlesia septentrionalis*.

SUMMARY

A new bacterial disease of *Bowlesia septentrionalis* is reported. By means of isolations and inoculations, the causal organism has been shown to be a new species of the genus *Phytomonas*. The cultural features, physiological and biological properties, and the morphological characteristics of the organism are given.

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